Applications of a Biorelevant *In Vitro* Dissolution Method Using USP Apparatus 4

in Early Phase Formulation Development

By

Vivian Ku Robertson

Submitted to the graduate degree program in Pharmaceutical Chemistry and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Master of Science.

_____

Chairperson John F. Stobaugh

_____

John I. Chung

_____

M. Laird Forrest

Date Defended: May 15, 2013

The Thesis Committee for Vivian Ku Robertson

certifies that this is the approved version of the following thesis:

Applications of a Biorelevant *In Vitro* Dissolution Method Using USP Apparatus 4

In Early Phase Formulation Development

_____

Chairperson John F. Stobaugh

Date approved: May 15, 2013

Abstract

Dissolution plays various roles throughout drug development, including assessment of the lot-to-lot quality of a drug product, guidance for development of new formulations, and assurance of continuing product quality and performance throughout a drug's lifecycle. To that end, one of the most important and useful applications of dissolution testing is to predict the *in vivo* performance of solid oral dosage forms.

However, there are several limitations of the traditional dissolution method that often emphasizes its quality control role with the primary objective to achieve 100% drug release, particularly during first in human trials. Some of these limitations include inadequate dissolution of poorly soluble drugs as well as the use of simple aqueous buffer solutions and hydrodynamics, which do not represent the *in vivo* environment.

The USP apparatus 4 in the open system configuration has more laminar hydrodynamics than other USP apparatuses. Together with the use of biorelevant dissolution media, this *in vitro* dissolution system may better mimic the *in vivo* environment, which may provide information that is clinically-relevant throughout clinical development. Using this system, an *in vitro* dissolution method was developed in a systematic way using the BCS class II compound, ibuprofen as the model compound.

This *in vitro* dissolution method was then applied to additional BCS class II compounds spanning a broad range of commercial and development compounds within this BCS class. Specifically, the work presented in this thesis suggests there are several potential applications for the *in vitro* biorelevant dissolution method developed. These applications include rank ordering of formulations, evaluation of pH modifiers, evaluation of food effect, evaluation of dose assessment, and lot-to-lot consistency.

Acknowledgements

First, I would like to thank John Chung my Amgen advisor. His mentorship and guidance was invaluable while writing my thesis, particularly his keen insight and expertise in the fields of drug absorption and simulations.

Additionally, this work could not have been possible without the guidance and support of my original Amgen advisor, Jiang (Jan) Fang. This thesis is as much an achievement for me as it is for Dr. Fang and I hope to continue to build the skills she has taught me throughout my career.

I would like to thank John Stobaugh, my KU advisor for his support throughout my masters experience at the University of Kansas. Additionally, my thesis committee would have not been complete without Laird Forrest. Thank you for your support and scientific discussion.

Archana Rawat, Tawnya Flick, and Zhe (Jean) J Tang must also be thanked for their contributions toward the early development work that helped to formulate the *in vitro* biorelevant dissolution test parameters for USP apparatus 4.

I would like to thank Judith (Judy) Ostovic, my current Amgen supervisor for her support, particularly during the writing process and her help with time management during these hectic times. I thank Angie Olsofsky, Charles Yang, and Cesar Medina provided Amgen development drug products. And also thank the AR&D directors for their support: Janet Cheetham, Andrew Clausen, Nina Cauchon, David Semin, and James McElvain.

Nancy Helm was paramount throughout this process. Her prompt help and kind attention to detail made me feel like part of the Pharmaceutical Chemistry KU family.

And finally, I would like to thank Christian Schoenich, John Stobaugh, and all the Pharmaceutical Chemistry staff at KU as well as Dave Brems and Janet Cheetham for supporting the KU Distance Learning Masters program.

Table of Contents

Chapter 1. Introduction

Importance of Dissolution

Pharmaceutical companies make a profitable business in developing drugs from the start of discovery of a new molecular target all the way through to filing, and approval. While these activities can take the better part of a quarter of a century to complete they are paramount to address the many ailments of man. From the simple headache to the complex, ever-enduring battle of cancer, each drug will target a different molecular pathway, using a elegantly matched dosage form to allow for an effective route of administration so that the drug can address the ailment it is indicated for.

With this in mind, the primary focus during preclinical and clinical development is the dosage form and how best to modify or formulate the drug to make a successful dosage form. While each dosage form is characterized by key attributes with distinct advantages and disadvantages related to drug development including ease of manufacturing, ease of dosing, and even patient compliance, for the purposes of this thesis work, solid dosage forms (i.e., tablets and capsules) are the primary focus of this work.

Using the solid oral dosage form as a reference, several things must occur before the pharmaceutical effects of a drug are experienced when it is administered orally to the patient. Using the commonly used over-the-counter drug Tylenol®, which is used to treat a headache or fever as an example, the drug absorption from the Tylenol® tablets after oral administration depends on several factors including:

(1) the release of the drug substance (acetaminophen) from the drug product (Tylenol® tablet),

(2) the dissolution or solubilization of the drug under physiological conditions, and

(3) the permeability of the drug across the gastrointestinal tract (GIT) (*1*).

It is also important to keep in mind that drug absorption and bioavailability are often significantly affected by the route of administration, dosage form, and co-administration of other substances, which have been major drivers of pharmaceutical research over the last two decades (*2*). And because of the important nature of the first two steps of oral administration described above (release of the drug substance from the drug product and dissolution or solubilization of the drug under physiological conditions), *in vitro* dissolution may be relevant to the prediction of *in vivo* performance (*1*).

In fact, *in vitro* dissolution tests for immediate release solid oral dosage forms are used to accomplish several objectives throughout drug development including:

1) assess the lot-to-lot quality of a drug product;

2) guide development of new formulations; and

3) ensure continuing product quality and performance after certain changes, such as changes in the formulation, the manufacturing process, the site of manufacture, and the scale-up of the manufacturing process (*1*).

Much work has been done to use *in vitro* dissolution as a quality control (QC) tool to ensure lot-to-lot consistency (*2-7*). Additionally, *in vitro* dissolution has been used as a surrogate for *in vivo* bioequivalence and *in vivo-in vitro* correlation (IVIVC) studies (*2-7*). Although used less frequently then its QC counterpart, *in vitro* dissolution can glean equally important information to guide formulation development.

Traditional Dissolution

In order for a drug to be absorbed *in vivo* it must be solubilized in the aqueous environment of the gastrointestinal tract (GIT) and for this reason the dissolution test for solid oral drug products has emerged as a critical control test for assuring product uniformity and batch-to-batch bioequivalence once the drug's bioavailability has been defined (*1, 8*). As a consequence the primary focus of *in vitro* dissolution tends to be its quality control applications, which typically target 100% drug release regardless of *in vivo* bioavailability.

To achieve this "traditional dissolution," some methods, including United States Pharmacopeia (USP) monograph methods use large amounts of surfactants, high pH, and even high levels of alcohol (*9*). Although such measures need to be justified these methods frequently are not biorelevant and applying such an *in vitro* dissolution method may be overdiscriminating, where *in vitro* dissolution differences are not seen *in vivo*, or not discriminating enough where there are no differences seen by *in vitro* dissolution when in fact they exist *in vivo.*

In addition, methods are commonly product-specific, where different strengths of the same formulation may use different media for testing. In such a case, results from one method may not necessarily be comparable to those of the other method so that comparison across strengths of the same formulation cannot be evaluated. Therefore, application of a traditional dissolution method in early phase drug product development is often limited due to limited clinical experience or poor *in vivo* correlations, making forecasting of *in vivo* drug performance extremely difficult.

Biorelevant Dissolution

Based on some of the limitations of tradition dissolution mentioned, it has been suggested that dissolution testing be carried out under physiological conditions. This allows interpretation of dissolution data with regard to *in vivo* performance of the product. The testing conditions should be based on physicochemical characteristics of the drug substance and the environmental conditions the dosage form might be exposed to after oral administration (*1*).

In order to properly mimic *in vivo* conditions in an *in vitro* environment, particular emphasis is made on dissolution media and hydrodynamics. Dissolution media can directly be addressed using critical biorelevant components in the *in vitro* dissolution method while hydrodynamics will be examined in the context of USP apparatuses.

Biorelevant Media

Biorelevant media is meant to mimic the physiological conditions in the gastrointestinal tract. In several cases, biorelevant media have been reported to facilitate the prediction of *in vivo* drug release (*10-18*). Specifically, there are four standard biorelevant dissolution media that are typically used in *in vitro* dissolution and they include:

(1) Simulated gastric fluid (SGF)

(2) Simulated intestinal fluid (SIF)

(3) Fasted state simulated intestinal fluid (FaSSIF)

(4) Fed state simulated intestinal fluid (FeSSIF)

See Table 1 for the corresponding composition for each biorelevant media used.

In brief, each media represents various pH and or components associated with the gastrointestinal tract with SGF representing the pH or components observed in the stomach (pH 1.2), SIF mimicking the intestinal tract (pH 6.8), and FaSSIF and FeSSIF mimicking the fasted

or fed conditions in the intestine, respectively, which may be applied to an *in vitro* biorelevant dissolution method.

Table 1. Biorelevant Dissolution Media Compositions (*15*)

Media	**Composition**
Simulated gastric fluid (SGF), pH 1.2	2 g Sodium chloride 3.2 g Purified pepsin (omitted) 7 mL Hydrochloric acid 1000 mL Water q.s.
Simulated intestinal fluid (SIF), pH 6.8	6.8 g Potassium phosphate monobasic 77 mL Sodium hydroxide (0.2 N) 10 g Pancreatin (omitted) 1000 mL Water q.s.
Fasted stated simulated intestinal fluid (FaSSIF), pH 6.8, Version 1	0.029 M Potassium phosphate monobasic pH 6.8 Sodium hydroxide q.s. 5 mM Sodium taurocholate 1.5 mM Lecithin 0.22 M Potassium chloride 1000 mL Water q.s.
Fed state simulated intestinal fluid (FeSSIF), pH 5.0, Version 1	0.144 M Acetic acid pH 5.0 Sodium hydroxide q.s. 15 mM Sodium taurocholate 4 mM Lecithin 0.19 M Potassium chloride 1000 mL Water q.s.

United States Pharmacopeia (USP) Dissolution Apparatuses (*13*)

There are several types of dissolution apparatus described in the USP:

1) USP apparatus 1: Basket

2) USP apparatus 2: Paddle

3) USP apparatus 3: Reciprocating cylinder

4) USP apparatus 4: Flow-through cell

5) USP apparatus 5: Paddle over disk

6) USP apparatus 6: Cylinder

7) USP apparatus 7: Reciprocating holder

USP apparatus 1 and 2 are the most frequently used, however, they do not necessarily mimic the conditions *in vivo*, particularly in terms of hydrodynamics. In contrast, USP apparatus 4 may have biorelevant applications because its flow is more laminar, less turbulent than other USP appartuses (*19*). Additionally, the USP 4 apparatus is well-suited for low solubility, high permeability compounds in the open system configuration. See Figures 1 and 2 for diagrams of USP apparatus 2 and USP apparatus 4, respectively.

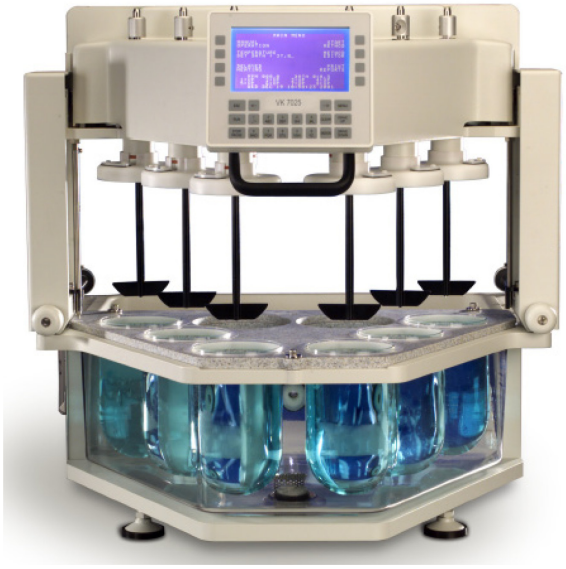

Figure 1. Diagram of USP apparatus 2 (*20*)

Figure 2. Diagram of USP apparatus 4 (*21*)

Due to the complexity of the human gastrointestinal tract (GIT), it is difficult to mimic *in vivo* hydrodynamics in an *in vitro* dissolution setting. The USP apparatus 4 in the open system configuration, however, offers some distinct advantages. See Figure 3 for a schematic of USP apparatus 4 open system.

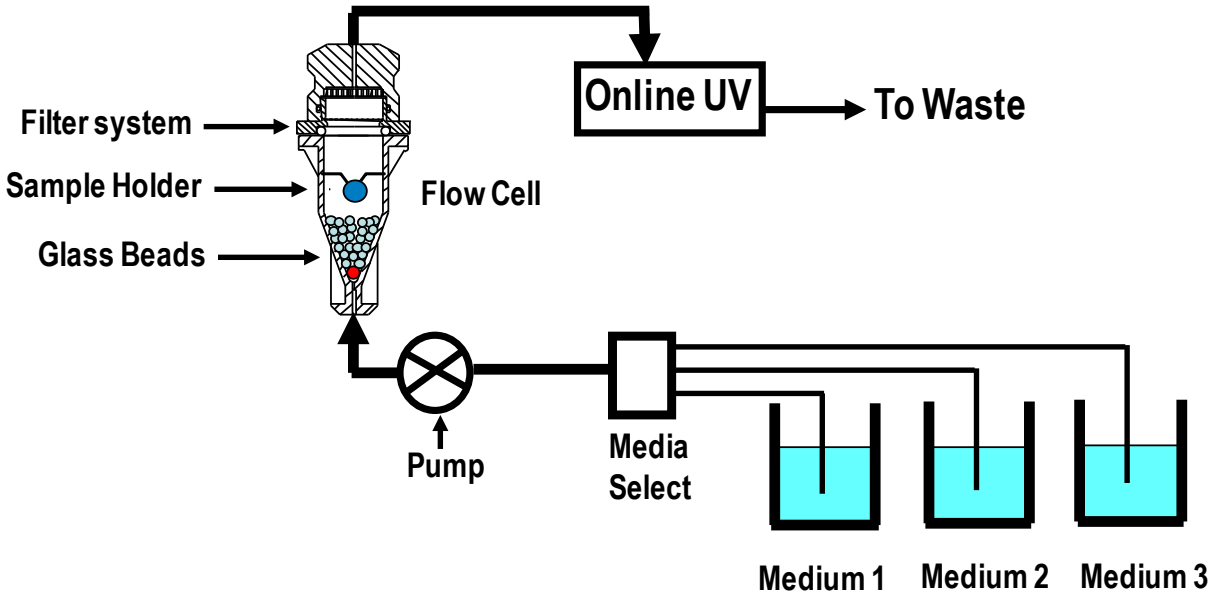

Figure 3. Diagram of USP apparatus 4 open system (*22*)

In this system, fresh solvent can continuously pass through the flow-cell to bring the dissolved material up and out of the cell, analogous to the way high permeability compounds

pass through the human GIT. This continuous introduction of fresh media allows the USP apparatus 4 open system configuration to consistently maintain sink conditions for a poorly soluble drug.

Additionally, the design of the pump, presence of the glass beads, and design of the flow-cell help control the flow of dissolution media with less turbulence as compared to other dissolution apparatuses. Therefore, the flow-through cell open system has the potential to better simulate *in vivo* hydrodynamics in an *in vitro* setting.

Overview of Thesis Work

One of the most important and commonly used applications of dissolution testing during drug development is to predict the *in vivo* performance of solid oral dosage forms. However, traditional dissolution often uses simple aqueous buffers in quality control-type methods and therefore rarely represents the physiological conditions in the human gastrointestinal tract. If the relevant *in vivo* conditions can be mimicked in an *in vitro* dissolution setting there may be an opportunity to predict the *in vivo* performance of solid oral dosage forms.

With this in mind, this thesis work will focus on leveraging this concept of biorelevant dissolution where the combination of biorelevant dissolution media and USP apparatus 4 in the open system configuration may adequately mimic the physiological conditions of the GIT. Therefore, this *in vitro* biorelevant dissolution testing may potentially predict the *in vivo* performance of a solid oral dosage form in a qualitative manner.

Chapter 2 describes the development of an *in vitro* biorelevant dissolution method using a systematic method development approach.

Chapter 3 explores some potential applications of the method through the following 5 case studies, which may ultimately aid formulation selection during drug development:

1) Rank ordering of development formulations
2) Effect of pH modifier
3) Assessment and prediction of food effect
4) Dose proportion
5) Lot-to-lot variability

Finally, Chapter 4 discusses the overall conclusions of this thesis work.

References

1. *Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Drug Dosage Forms*; U.S. Food and Drug Administration, Center of Drug Evaluation and Research (CDER): Rockville, MD, 1997.

2. Haidar, S. H.; Davit, B.; Chen, M. L.; Conner, D.; Lee, L. M.; Li, Q. H.; Lionberger, R.; Makhlouf, F.; Patel, D.; Schuirmann, D.; Xu, J.; Lawrence, X. Bioequivalence Approaches for Highly Variable Drugs and Drug Products, *Pharm. Res.* **2008**, *25* (1), 237-241.

3. Cardot, J. M.; Beyssac, E.; Airic, M. In Vitro-In Vivo Correlation: Importance of Dissolution in IVIVC, *Dissolution Technol.* **2007**, *14* (1), 15-19.

4. Dressman, J.; Amidon, G.; Reppas, C.; Shah, V. Dissolution Testing as a Prognostic Tool for Oral Drug Absorption: Immediate Release Dosage Forms, *Pharm. Res.* **1998**, *15* (1), 11-22.

5. Cheng, C. L.; Yu, L. X.; Lee, H. L.; Yang, C. Y.; Lue, C. S.; Chou, C. H. Biowaiver Extension Potential to BCS Class III High Solubility-Low Permeability Drugs: Bridging Evidence for Metformin Immediate-Release Tablet, *Eur. J. of Pharm. Sci.* **2004**, *22* (4), 297-304.

6. *Guidance for Industry: Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Dosage Forms Based on a Biopharmaceutics Classification System*; U.S. Food and Drug Administration, Center of Drug Evaluation and Research: Rockville, MD, 2000.

7. McGilveray, I. Overview of Workshop: *In Vitro* Dissolution of Immediate Release Dosage Forms: Development of *In Vivo* Relevance and Quality Control Issues, *Drug Info. J.* **1996**, *30*, 1029-1037.

8. Skelly, J. P.; Amidon, G. L.; Barr, W. H.; Benet, L. Z.; Carter, J. E.; Robinson, J. R.; Shah, V. P.; Yacobi, A. *In Vitro* and *In Vivo* Testing and Correlation for Oral Controlled/Modified-Release Dosage Forms, *Pharm. Res.* **1990**, *7*, 975-982.

9. FDA dissolution database, http://www.fda.gov

10. Kalantzi, L.; Person, E.; Polentarutti, B. S.; Abrahanmsson, B.; Goumas, K.; Dresman, J.; Reppas, C. Canine Intestinal Contents vs. Simulated Media for the Assessment of Solubility of Two Weak Bases in the Human Small Intestinal Contents. *Pharm. Res.* **2006**, *23* (6), 1373-1381.

11. Jantratid, E.; Janssen, N.; Reppas, C.; Dressman, J. Dissolution Media Simulating Conditions in the Proximal Human Gastrointestinal Tract: An Update. *Pharm. Res.* **2008**, *25* (7), 1663-1676.

12. Klein, S.; Butler, J.; Hemenstall, J.; Reppas, C.; Dressman, J. Media to Simulate Postprandial Stomach I. Matching the Physicochemical Characteristics of Standard Breakfasts. *J. Pharm. Pharmacol.* **2004**, *56*, 250-256.

13. *United States Pharmacoepia and National Formulary*, 26th and 30th eds.; United States Pharmacopeial Convention Inc.: Rockville, MD, 2003 and 2007.

14. Stippler, E.; Kopp, S.; Dressman, J. Comparison of US Pharmacopeia Simulated Intestinal Fluid TS (without Pancreatin) and Phosphate Standard Buffer pH 6.8, TS of the International Pharmacopeia with Respect to Their Use In Vitro Dissolution Testing. *Dissolution Technol.* **2004**, *11* (2), 6-10.

15. Galia, E.; Nicolaides, E.; Reppas, C.; Dressman, J. New Media Discriminate Dissolution Properties of Poorly Soluble Drugs. *Pharm. Res.* **1996**, *13*, 262-269.

16. Marques, M. Dissolution Media Simulating Fast and Fed States. *Dissolution Technol.* **2004**, *11* (1), 11-16.

17. Vertzoni, M.; Pastelli, E.; Pasachoulias, D.; Kalantzi, L.; Reppas, C. Estimation of Intragastric Solubility of Drugs: In What Medium. *Pharm. Res.* **2007**, *24* (5), 909-917.

18. Klein, S.; Dressman, J. Comparison of Drug Release from Metoprolol Modified Release Dosage Forms in Single Buffer Versus a pH-Gradient Dissolution Test. *Dissolution Technol.* **2006**, *13* (1), 6-12.

19. Fotaki, N.; Reppas, C. The Flow Through Cell Methodology in the Evaluation of Intralumenal Drug Release Characteristics. *Dissolution Techol.* **2005**, *12* (1), 17–21.

20. USP 2 diagram, http://www.varianinc.com/image/vimage/docs/products/dissolution/shared/SI-0784_VK_7025_DS_v05.pdf (accessed Aug 25, 2011)

21. USP 4 diagram, http://www.sotax.com/fileadmin/sps/Brochures/ce_7_flow_through_systems.pdf (accessed Aug 25, 2011)

22. Zolnik, B. S.; Raton, J-L.; Burgess, D. J. Applicaton of USP Apparatus 4 and In Situ Fiber Optic Analysis to Microsphere Release Testing, *Dissolution Technol.* **2005**, *12* (2), 11-14.

Chapter 2. Development of a Generic Biorelevant *In Vitro* Dissolution Method

Introduction

Dissolution testing plays many important roles in drug product development such as quality control (QC), predicting *in vivo* release, guiding formulation development, and establishing *in vivo-in vitro* correlation (IVIVC) to minimize *in vivo* studies (*1*). More specifically, there should be enough flexibility in the *in vitro* dissolution methodology to allow for development of methods that truly reflect the *in vivo* rate controlling process for a given drug; this is particularly important for a method that might be used as a surrogate for an *in vivo* bioavailability test (*2*).

However, the traditional dissolution approach strongly emphasizes QC applications and usually strives to obtain 100% drug release. As a result, the methods are not necessarily biorelevant (*3*) and quite often do not correspond to *in vivo* data, making forecasting of *in vivo* drug performance extremely difficult. Therefore, it is desirable to develop a biorelevant dissolution method to predict the rank order of formulation performance. Such a method may indicate a relationship or effect between food and *in vivo* drug release (*3*) and may help to establish or understand an IVIVC or an *in vivo-in vitro* relationship (IVIVR), which may facilitate the development of new drug products.

IVIVC and IVIVR has been vigorously attempted for more than four decades (*4-8*). Unfortunately, IVIVC and IVIVR cannot realisticially be applied to all drug products for various reasons (*4, 5, 8*) and typically is only applied to drugs with dissolution rate limited absorption. This is particularly true for immediate release products even though they are the most popular products on the market (*7*).

In order to properly utilize *in vitro* dissolution data to predict *in vivo* performance, it has been suggested that *in vitro* dissolution parameters, should mimic *in vivo* physiological conditions. Such parameters to consider include media composition, volume, hydrodynamics, duration of the test, and even analysis of the data. Unfortunately, these parameters are somewhat limited by our knowledge of the conditions in the gastrointestinal tract (*4*) making it difficult to understand the underlying factors that affect dissolution.

The modified USP apparatus 4, also known as flow-through cell dissolution (*9*) is the USP dissolution apparatus that most closely mimics *in vivo* hydrodynamics versus any other dissolution apparatus (*4*). In conjunction with biorelevant media, this *in vitro* dissolution system may adequately mimic *in vivo* conditions to help understand the most important factors for dissolution.

Instrumentation and Materials

The subsequent studies were conducted using a USP apparatus 4 system (Sotax CE 7 Smart semi-automated system, Sotax Corporation, Horsham, PA) along with an online UV fiber optic unit (Opt Diss Fiber Optic UV Spectrophotometer with an Opt Diss Flow Through Manifold for USP 4 (Distek, North Brunswick, NJ)). See Figure 1 for a schematic of USP apparatus 4/online UV system used throughout this study.

Two hundred milligram Advil tablets (Wyeth Consumer Healthcare) and Motrin® tablets (Ortho-McNeil-Janssen Pharmaceuticals) were purchased from Longs Pharmacy. Two hundred milligram danazol capsules (Barr Laboratories) and 15 mg and 30 mg Prevacid SoluTabs (TAP Pharmaceuticals) were purchased from Burt's Pharmacy for research purposes. All relevant

standards were purchased from USP and/or Sigma-Aldrich and prepared in ethanol (Pharmco-Aaper, 200 proof).

All Amgen development compounds were manufactured/developed and formulated at Amgen, Inc. PK and clinical data were obtained from internal Amgen development studies.

Simulated gastric fluid (SGF, pH 1.2, no pepsin), simulated intestinal fluid (SIF, pH 6.8, no pancreatin, fasted state simulated intestinal fluid (FaSSIF, pH 6.8) and fed state simulated intestinal fluid (FeSSIF, pH 5.0) were prepared by Amgen, Inc.

Figure 1. Diagram of USP apparatus 4

Method Development

The method development approach used in this work was different from traditional *in vitro* dissolution method development, in which the goal is to achieve 100% drug release, which may or may not take into account *in vivo* drug performance. Instead, the known *in vivo* plasma

profiles of several model compounds were used to guide the biorelevant dissolution method development in this study. As mentioned, biorelevant dissolution media was used throughout method development and will be discussed in detail in a separate section. Together with the USP 4 apparatus, this *in vitro* dissolution system is meant to mimic the *in vivo* physiological environment as far as pH and hydrodynamics, which may result in more relevant *in vitro* dissolution conditions. If this is the case, particularly for highly permeable compounds, dissolution may be evaluated in a generic fashion, which more closely mimics physiological conditions. Additionally, *in vitro* biorelevant dissolution may allow method development to directly focus on *in vivo* versus *in vitro* profile comparisons and therefore may have more predictive power for *in vivo* drug release.

Gastrointestinal Tract (GIT)

Every dosage form that is administered orally will transit through the gastrointestinal tract (GIT). As the solid oral dosage form travels throughout the GIT, it will undergo absorption. Additionally, the solid oral dosage form will encounter varying pHs, transit times, and permeabilities associated with the different parts of the GI tract for healthy subjects. Strong acidic conditions are seen in the stomach, whereas higher pHs are seen as transit continues towards the intestine. pH will vary significantly depending on the presence of food. Some drugs will affected by the presence or absence of food while others will not. See Table 1 for additional details on the GIT (*10*).

Table 1. Additional Details of the Gastrointestinal Tract

	pH	Transit Time (hours)	Permeabilities
Stomach	1-3	0.5	Varies
Duodenum	4-6	-	Varies
Jejunum	6-7	-	Varies
Ileum	6-7	-	Varies
Small Intestine	-	3-5	Varies
Colon	5-7	25	Varies

Biopharmaceutics Classification System (BCS)

Before describing the model compounds used through this work some background information regarding the biopharmaceutics classification system (BCS) is appropriate. Specifically, Amidon proposed a biopharmaceutics drug classification scheme for correlating in vitro drug product dissolution and in vivo bioavailability based on fundamental parameters that control the rate and extent of drug absorption, namely drug solubility and gastrointestinal permeability (*2*).

The Biopharmaceutics Classification System (BCS) is routinely used by the Food and Drug Administration (FDA) to classify drugs based on solubility and permeability. The BCS categorizes drugs into one of four categories based on the solubility of the drug at its highest dose in 250 mL of buffer adjusted between pH 1.0 and 7.5 and its permeability determined either *in vivo* or experimentally, which is also described in Figure 2:

Case 1: High solubility, high permeability drugs

Case 2: Low solubility, high permeability drugs

Case 3: High solubility, low permeability drugs

Case 4: Low solubility, low permeability drugs

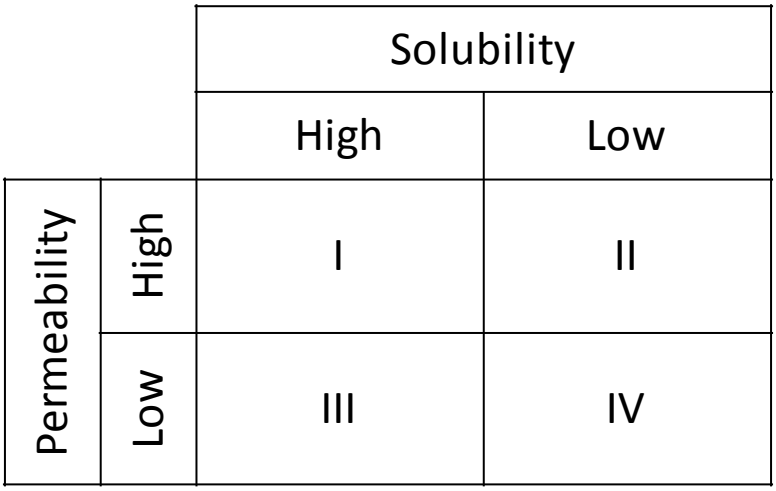

Figure 2. BCS Classification System (2)

Based on this classification system, the bioavailability of Class I drugs (high solubility, high permeability) and to a certain extent Class III drugs (high solubility, low permeability) is not limited by dissolution, but rather gastric emptying since drug dissolution tends to be so rapid (*1, 2*). In contrast, Class II drugs (low solubility, high permeability) may be limited by the drug's dissolution and are often referred to as "dissolution or solubility-limited drugs" while Class IV drugs (low solubility, low permeability) present significant challenges for oral drug delivery. This classification system also helps to guide in vitro dissolution specifications and provides a basis for correlating *in vivo* and *in vitro* data (*1*).

Model Compounds

Based on the BCS, Class II compounds have low solubility and high permeability. In terms of drug release or absorption, dissolution of BCS Class II compounds is often the rate-determining step. It is for this reason that BCS Class II compounds were selected for initial evaluation in the development of this *in vitro* dissolution method. In this context, BCS Class II compounds should minimize the impact from permeability throughout method development and also leverage the fact that dissolution is the rate-limiting step of drug release or absorption.

Several commercially available BCS class II compounds with known *in vivo* plasma profiles were used as model compounds to guide the biorelevant dissolution method development.

In order to evaluate the dissolution behavior of the model compounds systematically they were divided into the following three categories:

(1) Acidic compounds

(2) Basic compounds

(3) Neutral compounds

See Table 2 for the BCS class II model compounds used throughout method development. Table 3 includes additional details on the model compounds.

Table 2. BCS Class II Model Compounds

Acidic Compounds	**Neutral Compounds**	**Basic Compounds**
Ibuprofen (Advil, Motrin)	Carbamazepine (Tegretol)	Ketoconazole (Nizoral)
Naproxen (Naprosyn, Naprelan)	Danazol	Lansoprazole (Prevacid)
Warfarin (Coumadin)	-	Raloxifene (Evista)

Table 3. Additional Details on BCS Class II Model Compounds

Active Ingredient	Acidic /Basic /Neutral	pKa(s)[11]	Aqueous Solubility (μg/mL)[11]	Strength & Brand Name	Manufacturer
Ibuprofen	Acidic	4.91	49	200 mg Advil® 200 mg Motrin®	Wyeth Consumer Ortho-McNeil-Janssen
Naproxen	Acidic	4.15	15.9	500 mg Naprosyn 500 mg Naprelan	Roche Laboratories Hi Tech Pharmacal
Warfarin	Acidic	5.08	17	2, 5, 10 mg Coumadin	Bristol-Myers Squibb
Carbamazepine	Neutral	-	17.7	200 mg Tegretol	Novartis
Danazol	Neutral	-	0.0176	200 mg danazol	Barr
Ketoconazole	Basic	-	0.0866	200 mg Nizoral® ketoconazole	Mylan
Lansoprazole	Basic	17.3	0.97	15, 30 mg Prevacid SoluTabs	TAP
Raloxifene	Basic	9.55	0.25	60 mg Evista	Eli Lilly

Of these model compounds a variety of salt forms (e.g., free base, salt, etc.) and types of dosage forms (immediate-release tablets, enteric-coated tablets, and sustained release products) were used throughout method development and subsequent testing of the *in vitro* biorelevant dissolution method.

Ibuprofen (both Advil® and Motrin® tablets), was the primarily model compound used throughout method development (*12, 13*) where the resulting method was used to run all other model compounds and development dosage forms.

Biorelevant Media

In vivo solubilization is a critical consideration during development of an *in vitro* dissolution method and the dissolution media should reflect the *in vivo* situation (*14*) which allows for interpretation of the dissolution data while keeping the *in vivo* performance of the

product in mind. The testing conditions should be based on physicochemical characteristics of the drug substance and the environmental conditions the dosage form might be exposed to after oral administration (*1*). In this context, various biorelevant media that mimic the physiological conditions in the gastrointestinal tract have been reported to facilitate the prediction of *in vivo* drug release (*15-22*).

Four standard biorelevant dissolution media were used in this study:

(1) Simulated gastric fluid (SGF)

(2) Simulated intestinal fluid (SIF)

(3) Fasted state simulated intestinal fluid (FaSSIF)

(4) Fed state simulated intestinal fluid (FeSSIF)

See Table 4 for the corresponding composition for each biorelevant media used.

In brief, each media represents various pH and or components associated with the gastrointestinal tract. SGF represents the pH or components observed in the stomach (pH 1.2) without enzymes, SIF mimics the intestinal tract (pH 6.8) without enzymes or bile salts, and FaSSIF and FeSSIF mimics the fasted or fed conditions in the intestine, respectively.

For *in vitro* testing purposes, the four media described were primarily used without enzymes, however, if needed enzymes should be evaluated on a case-by-case basis with adequate justification (*1*). No other media, organic solvents, or surfactants were used throughout dissolution testing although such components may be commonly used in traditional *in vitro* dissolution testing.

Table 4. Biorelevant Dissolution Media Compositions (*20*)

Media	Composition
Simulated gastric fluid (SGF), pH 1.2	2 g Sodium chloride 3.2 g Purified pepsin (omitted) 7 mL Hydrochloric acid 1000 mL Water q.s.
Simulated intestinal fluid (SIF), pH 6.8	6.8 g Potassium phosphate monobasic 77 mL Sodium hydroxide (0.2 N) 10 g Pancreatin (omitted) 1000 mL Water q.s.
Fasted stated simulated intestinal fluid (FaSSIF), pH 6.8, Version 1	0.029 M Potassium phosphate monobasic pH 6.8 Sodium hydroxide q.s. 5 mM Sodium taurocholate 1.5 mM Lecithin 0.22 M Potassium chloride 1000 mL Water q.s.
Fed state simulated intestinal fluid (FeSSIF), pH 5.0, Version 1	0.144 M Acetic acid pH 5.0 Sodium hydroxide q.s. 15 mM Sodium taurocholate 4 mM Lecithin 0.19 M Potassium chloride 1000 mL Water q.s.

Systematic Method Development

As previously mentioned, representative BCS Class II compounds from acidic, neutral, and basic categories were used to carry out systematic method development. Various parameters were evaluated during dissolution method development including:

(1) Flow rate: 2 mL/min – 20 mL/min

(2) Flow-through cell size: 12 mm inner diameter, 22.6 mm inner diameter

(3) Sample holder: Absence or presence of sample holder in flow-through cell

(4) Biorelevant dissolution media: SGF, SIF, FaSSIF, and FeSSIF

(5) Enzymes: Absence or presence of enzymes in dissolution medium

Some parameters were not varied throughout dissolution method development. These parameters include the following:

(1) Glass beads: 1 mm

(2) Filter pore size: 0.7 μm

Just as the solid oral dosage form encounters both the stomach and intestine after oral administration, the necessity of using SGF first and then changing the media to SIF to mimic the pH gradient in the gastrointestinal tract was also evaluated during the development.

The development work was largely conducted using 200 mg Advil and Motrin tablets (*1, 2*) with other model compounds used for confirmation and comparison purposes. Online UV data was collected using a product specific wavelength in each case. The subsequent data was then overlaid with the plasma concentration data each plotted against their own axes with each axis scaled to line-up the *in vitro* C_{max} and t_{max} with the respective portions of the *in vivo* data.

Method Development Optimization

Flow rate was the first parameter evaluated using the 22.6 mm inner diameter flow-through cell and SIF as the dissolution medium. Flow rates ranged from 2 mL/min to 20 mL/min. See Figures 3, 4, 5, and 6 for dissolution profiles at 4 mL/min, 6 mL/min, 8 mL/min, and 20 mL/min, respectively.

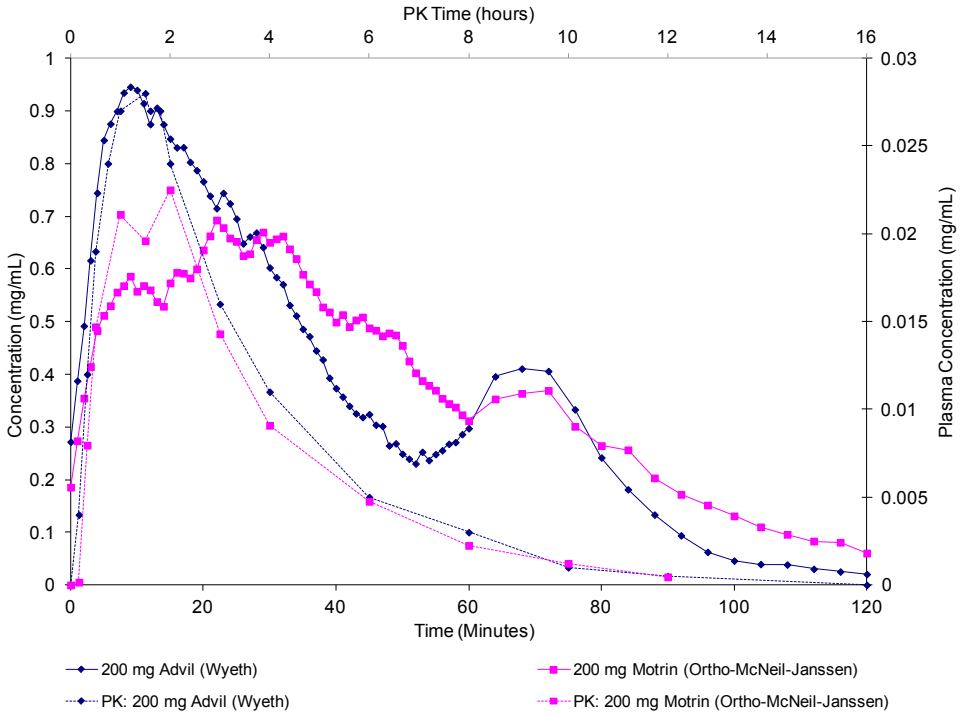

Figure 3. Plasma Profiles and *In Vitro* Dissolution Profiles of 200 mg Advil® and Motrin® at 4 mL/min using 22.6 mm Inner Diameter Flow-Through Cell in SIF

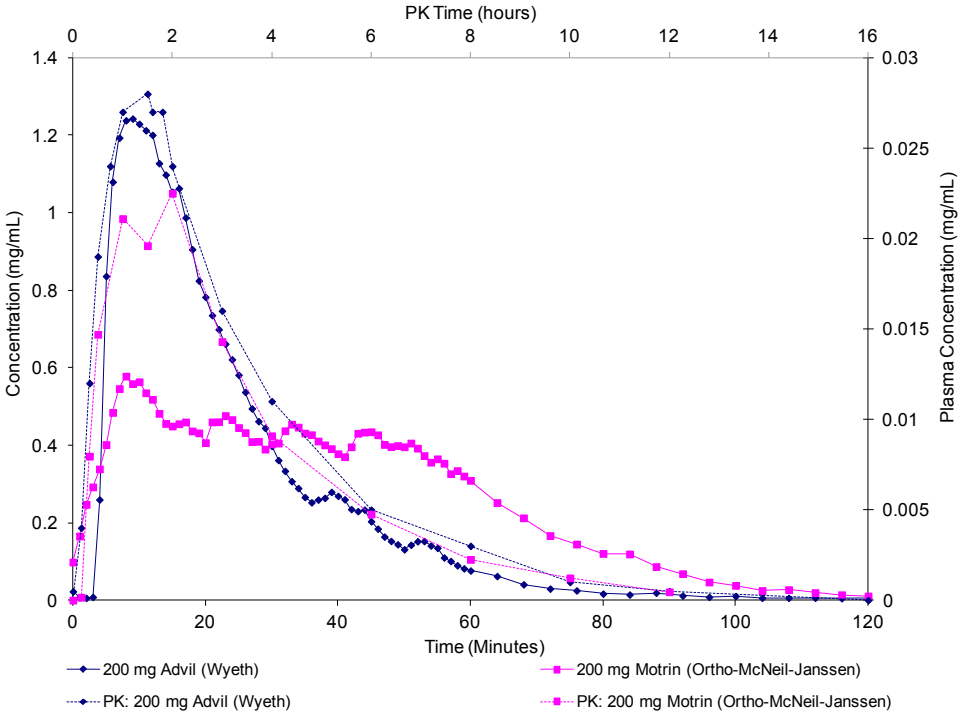

Figure 4. Plasma Profiles and *In Vitro* Dissolution Profiles of 200 mg Advil® and Motrin® at 6 mL/min using 22.6 mm Inner Diameter Flow-Through Cell in SIF

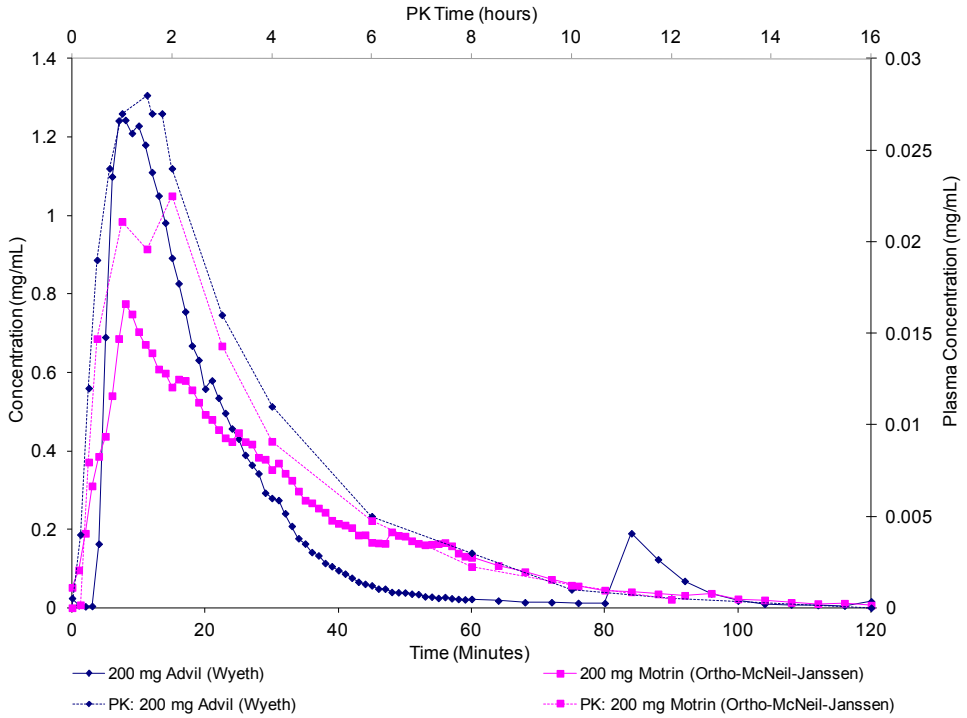

Figure 5. Plasma Profiles and *In Vitro* Dissolution Profiles of 200 mg Advil® and Motrin® at 8 mL/min using 22.6 mm Inner Diameter Flow-Through Cell in SIF

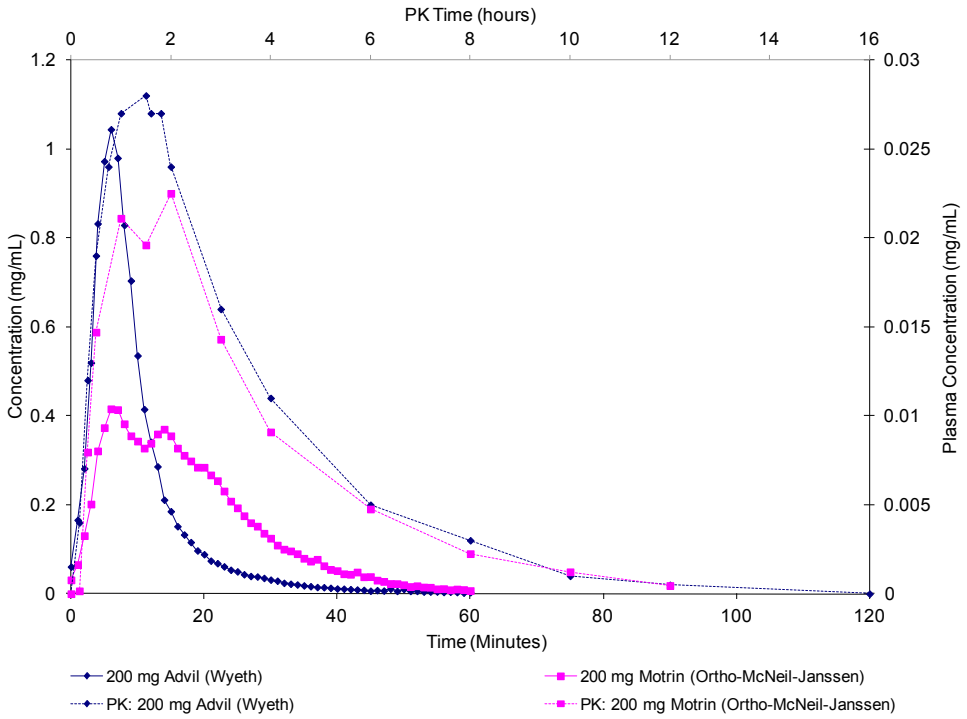

Figure 6. Plasma Profiles and *In Vitro* Dissolution Profiles of 200 mg Advil® and Motrin® at 20 mL/min using 22.6 mm Inner Diameter Flow-Through Cell in SIF

Results indicated that flow rate changes within this range led to the same rank ordering of drug release profiles. The appearance of the dissolution profiles, however, varied slightly as the flow rate changed, with sharper profiles observed for the higher flow rates and "flattened" profiles seen for slower flow rates. When the flow rate was at or below 6 mL/min, the resulting curves were more erratic with much noisier UV readings. A similar observation was noted when the small flow cell (12 mm inner diameter) was used, which might be attributed to the reduced homogeneity of the hydrodynamic flow in the system. See Figures 7 and 8 for dissolution profiles using 12 mm and 22.6 mm inner diameter flow-through cells, respectively.

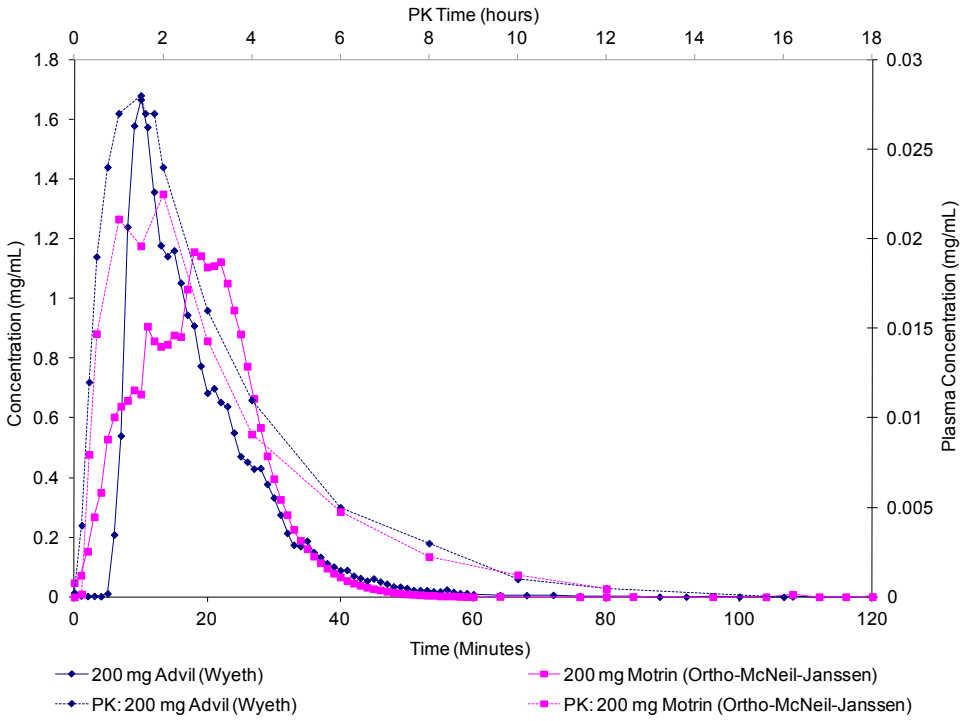

Figure 7. Plasma Profiles and *In Vitro* Dissolution Profiles of 200 mg Advil® and Motrin® at 8 mL/min using 12 mm Inner Diameter Flow-Through Cell in SIF

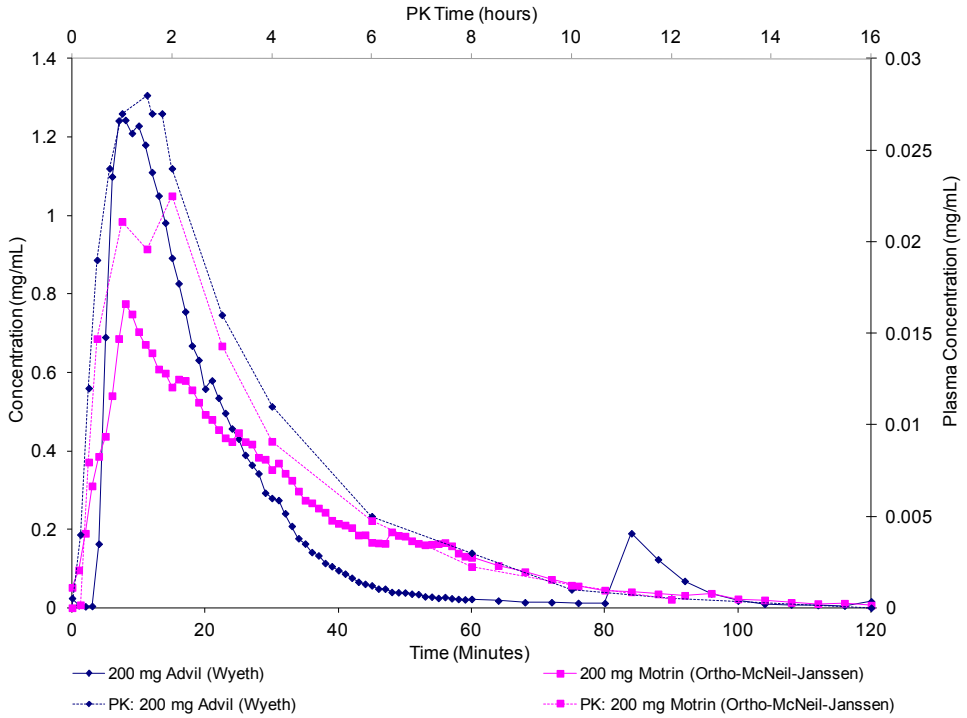

Figure 8. Plasma Profiles and *In Vitro* Dissolution Profiles of 200 mg Advil® and Motrin® at 8 mL/min using 22.6 mm Inner Diameter Flow-Through Cell in SIF

The need for SGF followed by a switch to a simulated intestinal fluid (e.g., SIF, FaSSIF, or FeSSIF), which mimics the pH gradient in the gastrointestinal tract was also evaluated during method development. In this testing, SGF and SIF were used as the dissolution test media. See Figures 9 and 10 for relevant dissolution profiles.

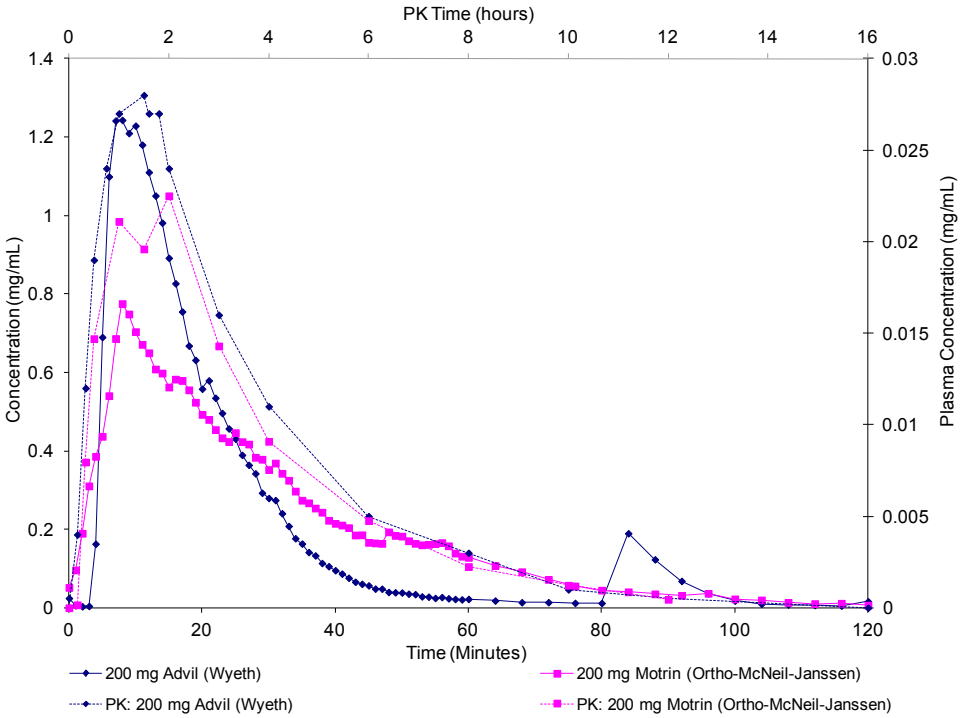

Figure 9. Plasma Profiles and *In Vitro* Dissolution Profiles of 200 mg Advil® and Motrin® at 8 mL/min using 22.6 mm Inner Diameter Flow-Through Cell in SIF (120 minutes)

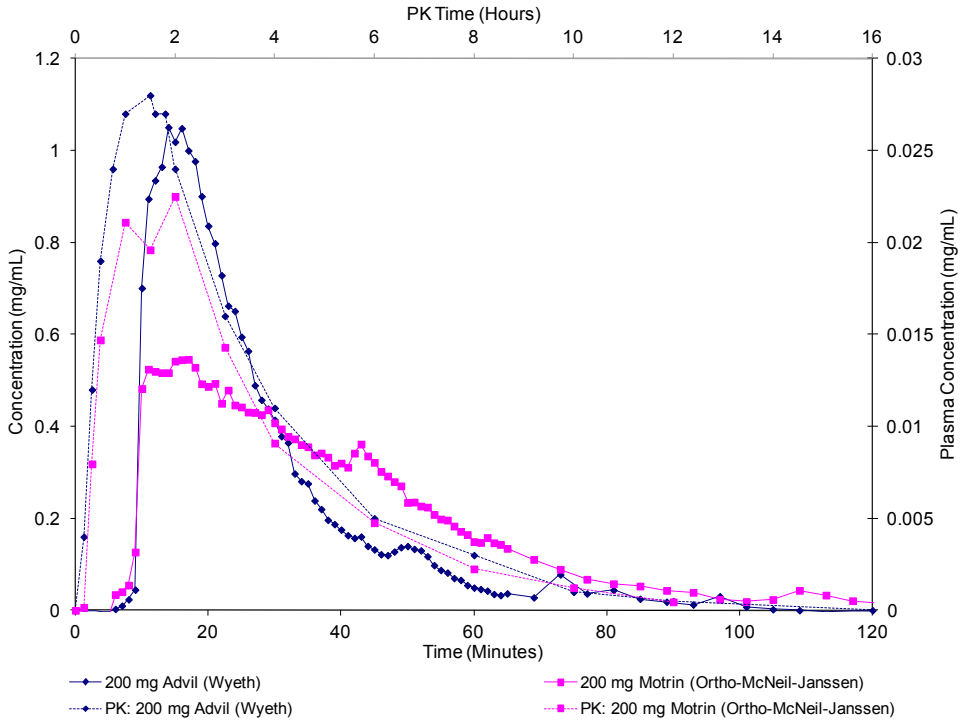

Figure 10. Plasma Profiles and *In Vitro* Dissolution Profiles of 200 mg Advil® and Motrin® at 8 mL/min using 22.6 mm Inner Diameter Flow-Through Cell in SGF (5 minutes) then SIF (120 minutes)

The results indicated that, for the acidic and neutral compounds, SIF may be used directly for the entire experiment instead of starting with SGF followed by a switch to a simulated intestinal fluid. The reason is that the rank order and profiles remain the same in both cases and therefore SGF provides no additional information and or discrimination for this *in vitro* dissolution test method.

However, for many of the basic compounds, the use of SGF is needed before switching to a simulated intestinal fluid. For drug products where disintegration rather than dissolution is the rate-determining step, the mid-run media switch should probably be considered as well to help break up the dosage form and encourage drug dissolution. Additionally, in some instances drug solubility may improve in the presence of FaSSIF or FeSSIF versus SIF and therefore may be

substituted as appropriate for poorly soluble drugs. One additional consideration when selecting media for capsule dosage forms is the need for enzymes and/or bile salts when cross-linking is evident. While cross-linking is not typically an issue *in vivo*, it is a very important factor to understand when applying an *in vitro* dissolution method because the dissolution of cross-linked capsules can be severely hindered in the absence of enzymes.

It was also observed that when an 8 mL/min flow rate was used, a five min hold time in SGF prior to switching to a simulated intestinal fluid provided a better match of the *in vivo* profiles yet maintained the appropriate rank order of the model compounds tested. When a significantly longer duration (e.g., 30 min) was used for SGF before the medium was switched to SIF, the discriminating power was lost and an *in vitro* "drug release burst" was observed. See Figures 11 and 12 for relevant dissolution profiles.

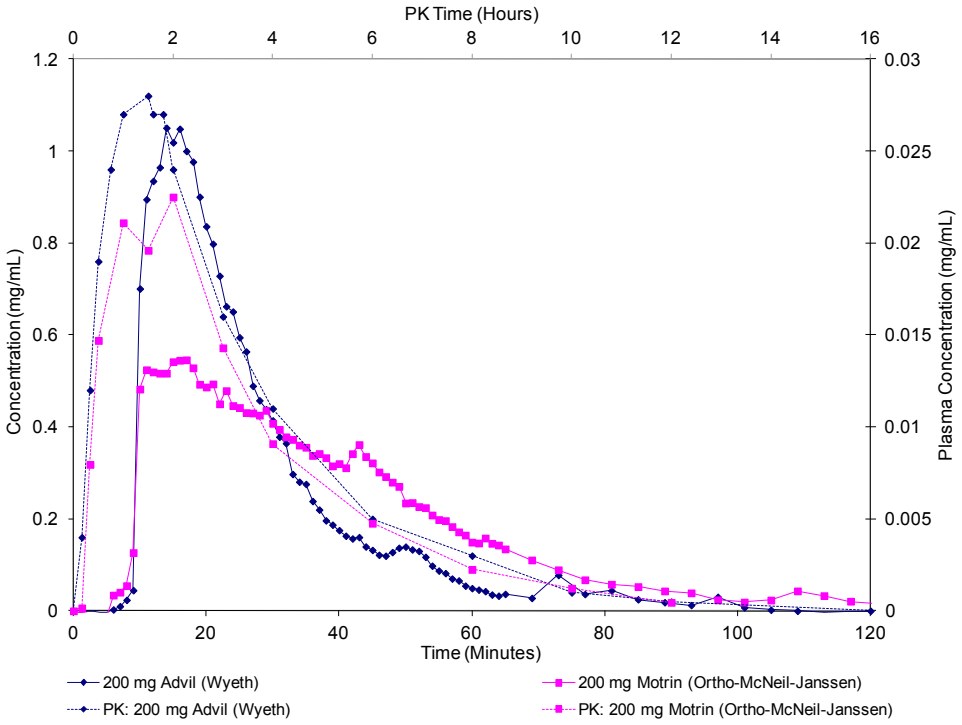

Figure 11. Plasma Profiles and *In Vitro* Dissolution Profiles of 200 mg Advil® and Motrin® at 8 mL/min using 22.6 mm Inner Diameter Flow-Through Cell in SGF (5 minutes) then SIF (120 minutes)

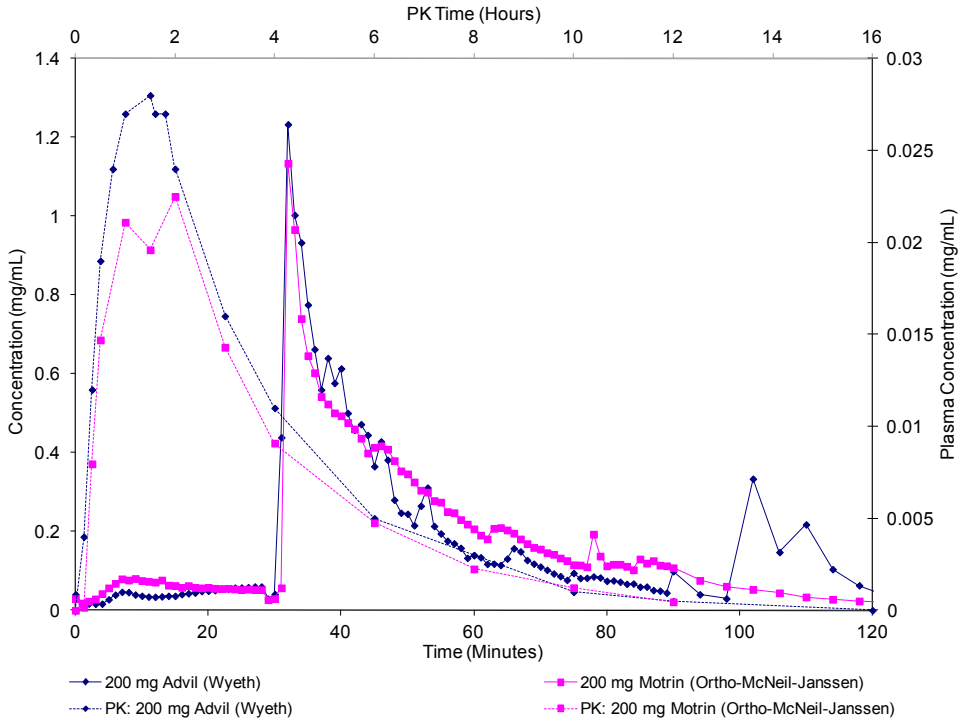

Figure 12. Plasma Profiles and *In Vitro* Dissolution Profiles of 200 mg Advil® and Motrin® at 8 mL/min using 22.6 mm Inner Diameter Flow-Through Cell in SGF (30 minutes) then SIF (120 minutes)

Rate Profiles versus Cumulative Profiles

Traditional dissolution data are often reported as cumulative profiles where percent dissolved is described in terms of time (i.e., % dissolved vs. time), whereas pharmacokinetic (PK) data are often reported as plasma concentrations described in terms of time. Recently, a study was performed in which the dissolution results were presented as concentration vs. time profiles in order to facilitate direct qualitative comparison between *in vivo* and *in vitro* profiles (*25*).

In this body of work, concentration vs. time profiles were routinely collected to examine the potential for direct qualitative comparison between *in vivo* and *in vitro* data. Cumulative profiles (% dissolved vs. time) were also calculated to monitor the overall drug dissolved in the system.

Method Development Summary

As a result of the systematic method development described above, the following conditions were selected:

(1) Flow rate: 8 mL/min

(2) Flow-through cell size: 22.6 mm inner diameter

(3) Sample holder: Presence of sample holder in flow-through cell

(4) Biorelevant dissolution media: Various as needed (SGF, SIF, FaSSIF, and FeSSIF)

(5) Enzymes: Absence of enzymes in dissolution medium

(6) Glass beads: 1 mm

(7) Filter pore size: 0.7 μm

Note that selection of a specific biorelevant dissolution media depends on the purpose of the study. For example, if the evaluation of the food effect is the main objective of the study, FaSSIF or FeSSIF should be used instead of the SIF. When both SGF and SIF media were used (i.e., switch from SGF to SIF), a hold time of 5 min was used for the initial SGF condition. One mm glass beads and a filter with 0.7 μm pore size were routine used. Glass wool was used to reduce backpressure when needed. All analyses were conducted using online UV detection with an appropriate UV wavelength that was compound-dependent. See Table 5 for the method development summary and Figure 13 for a representative dissolution profile.

Table 5. Biorelevant Dissolution Method Development Summary

Parameter	**Evaluated Condition(s)**	**Final Method**
Flow rate	2, 4, 6, 8, 16, and 20 mL/min	8 mL/min
Size of cell	12 or 22.6 mm inner diameter	22.6 mm inner diameter
Sample holder	Absence or presence of sample holder in flow-through cell	Presence
Biorelevant medium	Simulated gastric fluid (SGF) Simulated intestinal fluid (SIF) Fasted state simulated intestinal fluid (FaSSIF) Fed state simulated intestinal fluid (FeSSIF)	Various as needed
Enzyme	Absence or presence of enzymes in dissolution media	Absence
Glass beads	1 mm	1 mm
Filter pore size	0.7 µm	0.7 µm

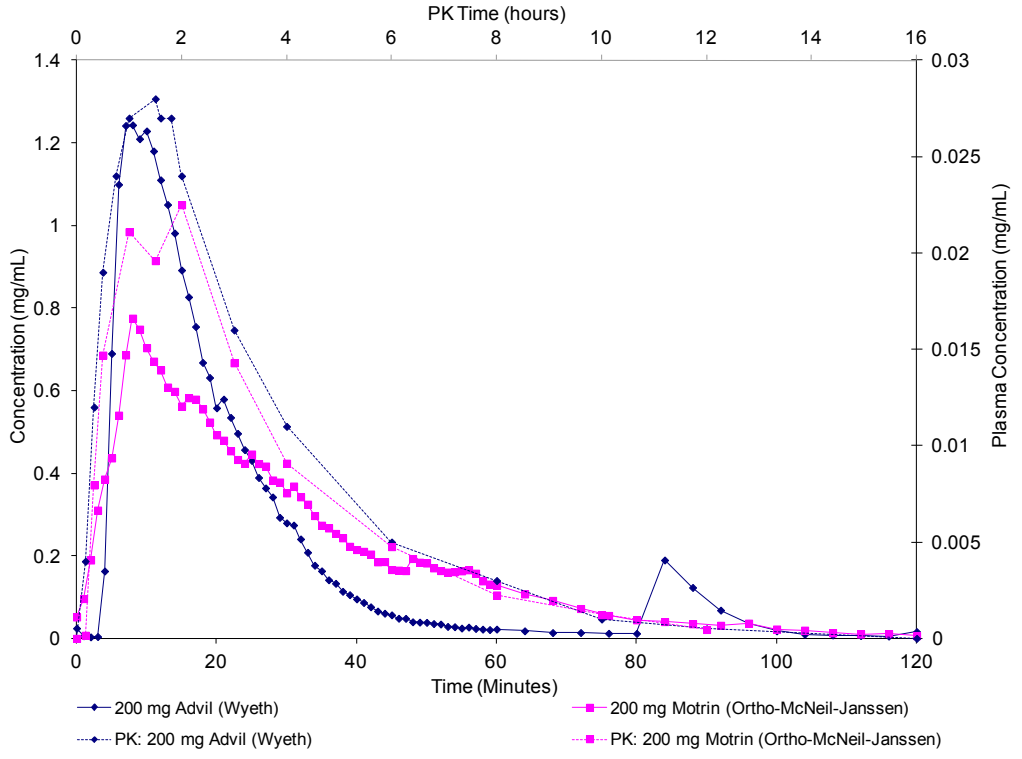

Figure 13. Plasma Profiles and *In Vitro* Dissolution Profiles of 200 mg Advil® and Motrin® at 8 mL/min using 22.6 mm Inner Diameter Flow-Through Cell in SIF (120 minutes)

The rate profile (concentration vs. time) was collected real-time and the cumulative profile (% dissolved vs. time) calculated later if needed. The resulting method was used directly without further product-specific development for all applications described.

Once the *in vitro* biorelevant dissolution method using USP apparatus 4 was developed, additional BCS class II drugs from Table 3 were tested for confirmatory purposes. For example, see Figure 14 for the *in vitro* biorelevant dissolution data for 200 mg Nizoral® ketoconazole tablets.

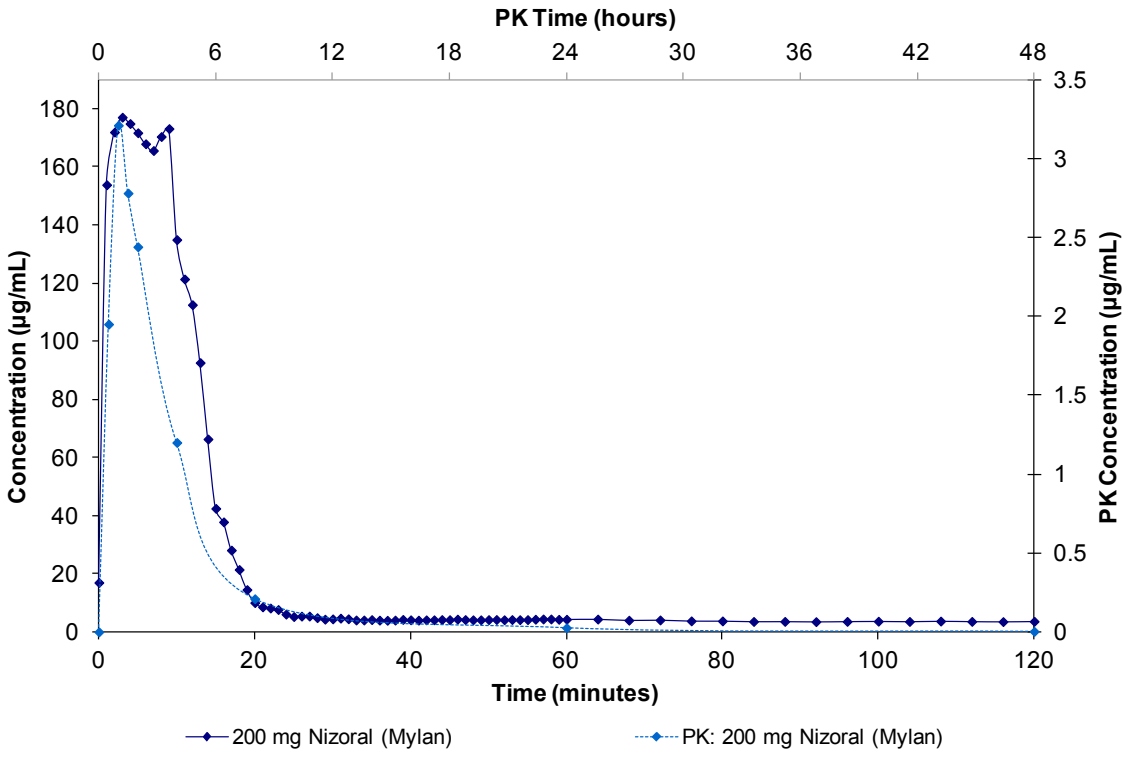

Figure 14. Plasma Profile and *In Vitro* Dissolution Profile of 200 mg Nizoral® Ketoconazole

References

1. *Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Drug Dosage Forms*; U.S. Food and Drug Administration, Center for Drug Evaluation and Research (CDER): Rockville, MD, 1997.

2. Amidon, G.; Lennernas, H.; Shah, V.; Crison, J. A Theoretical Basis for a Biopharmaceutic Drug Classification: The Correlation of *In Vitro* Drug Product Dissolution and *In Vivo* Bioavailability. *Pharm. Res*. **1995**, *12* (3), 413-420.

3. Nicolaides, E.; Galia, E.; Efthymiopoulos, C.; Dressman, J.; Reppas, C. Forecasting the *In Vivo* Performance of Four Low Solubility Drugs From Their *In Vitro* Dissolution Data. *Pharm. Res.* **1999**, *16*, 1876-1882.

4. Dressman, J.; Amidon, G.; Reppas, C.; Shah, V. Dissolution Testing as a Prognostic Tool for Oral Drug Absorption: Immediate Release Dosage Forms, *Pharm. Res.* **1998**, *15* (1), 11-22.

5. Galia, E.; Nicolaides, E.; Ho¨rter, D.; Lo¨benberg, R.; Reppas, C.; Dressman, J. Evaluation of Various Dissolution Media for Predict *In Vivo* Performance of Class I and II Drugs. *Pharm. Res.* **1998**, *15* (5), 698-705.

6. Cardot, J. M.; Beyssac, E.; Airic, M. In Vitro-In Vivo Correlation: Importance of Dissolution in IVIVC, *Dissolution Technol.* **2007**, *14* (1), 15-19.

7. Polli, J. E. IVIVR versus IVIVC. *Dissolution Techol.* **2000**, *7* (3), 6.

8. Meyer, M. C.; Straughn, A. B.; Mhatre, R. M.; Shah, V. P.; Williams, R. L.; Lesko, L. J. Lack of *In Vivo/In Vitro* Correlations for 50 mg and 250 mg Primidone Tablets. *Pharm. Res.* **1998**, *15*, 1085-1089.

9. Siewart, M.; Dressman, J.; Brown, C.; Shah, V. FIP/AAPS Guidelines for Dissolution/In Vitro Release Testing of Novel/Special Dosage Forms. *Dissolution Techol.* **2003**, *10* (1), 6-8, 10-13, 15.

10. Klein, S. Dissolution Tests to Predict Bioavailability of Drugs; *5th International Workshop on Physical Characterization of Pharmaceutical Solids,* June 24, 2004, Ettlingen, Germany.

11. Schettler, T.; Paris, S.; Pellett, M.; Kidner, S.; Wilkinson, D. Comparative Pharmacokinetics of Two Fast-Dissolving Oral Ibuprofen Formulations and Regular Release Ibuprofen Tablet in Healthy Volunteers. *Clin. Pharmacokinet.* **2001**, *21* (3), 73-78.

12. www.drugbank.ca

13. Gillespie, W. R.; Disanto, A. R.; Monovich, R. E.; Albert, K. S. Relative Bioavailability of Commercially Available Ibuprofen Oral Dosage Forms in Humans. *J. Pharm. Sci.* **1982**, *71* (9), 1034–1038.

14. Haidar, S. H.; Davit, B.; Chen, M. L.; Conner, D.; Lee, L. M.; Li, Q. H.; Lionberger, R.; Makhlouf, F.; Patel, D.; Schuirmann, D.; Xu, J.; Lawrence, X. Bioequivalence Approaches for Highly Variable Drugs and Drug Products, *Pharm. Res.* **2008**, *25* (1), 237-241.

15. Kalantzi, L.; Person, E.; Polentarutti, B. S.; Abrahanmsson, B.; Goumas, K.; Dresman, J.; Reppas, C. Canine Intestinal Contents vs. Simulated Media for the Assessment of Solubility of Two Weak Bases in the Human Small Intestinal Contents. *Pharm. Res.* **2006**, *23* (6), 1373-1381.

16. Jantratid, E.; Janssen, N.; Reppas, C.; Dressman, J. Dissolution Media Simulating Conditions in the Proximal Human Gastrointestinal Tract: An Update. *Pharm. Res.* **2008**, *25* (7), 1663-1676.

17. Klein, S.; Butler, J.; Hemenstall, J.; Reppas, C.; Dressman, J. Media to Simulate Postprandial Stomach I. Matching the Physicochemical Characteristics of Standard Breakfasts. *J. Pharm. Pharmacol.* **2004**, *56*, 250-256.

18. *United States Pharmacopeia and National Formulary*, 26th and 30th eds.; United States Pharmacopeial Convention Inc.: Rockville, MD, 2003 and 2007.

19. Stippler, E.; Kopp, S.; Dressman, J. Comparison of US Pharmacopeia Simulated Intestinal Fluid TS (without Pancreatin) and Phosphate Standard Buffer pH 6.8, TS of the International Pharmacopeia with Respect to Their Use In Vitro Dissolution Testing. *Dissolution Technol.* **2004**, *11* (2), 6-10.

20. Galia, E.; Nicolaides, E.; Reppas, C.; Dressman, J. New Media Discriminate Dissolution Properties of Poorly Soluble Drugs. *Pharm. Res.* **1996**, *13*, 262-269.

21. Marques, M. Dissolution Media Simulating Fast and Fed States. *Dissolution Technol.* **2004**, *11* (1), 11-16.

22. Vertzoni, M.; Pastelli, E.; Pasachoulias, D.; Kalantzi, L.; Reppas, C. Estimation of Intragastric Solubility of Drugs: In What Medium. *Pharm. Res.* **2007**, *24* (5), 909-917.

23. Klein, S.; Dressman, J. Comparison of Drug Release from Metoprolol Modified Release Dosage Forms in Single Buffer Versus a pH-Gradient Dissolution Test. *Dissolution Technol.* **2006**, *13* (1), 6-12.

24. Nicolaides, E.; Hempenstall, J. M.; Reppas, C. Biorelevant Dissolution Tests with the Flow-Through Apparatus? *Dissolution Techol.* **2000**, *7* (1), 8.

25. Carino, S.; Sperry, D.; Hawley, M. Relative Bioavailability of Three Different Solid Forms of PNU-141659 as Determined with the Artificial Stomach-Duodenum Model. *J. Pharm. Sci.* **2010**, *99* (9), 3923–3930.

Chapter 3. Applications of the *In Vitro* Biorelevant Dissolution Method Using USP Apparatus 4

Introduction

As described in Chapter 3, an *in vitro* biorelevant dissolution method was developed using USP apparatus 4 and commercially-available BCS class II compounds with known *in vivo* profiles. Ibuprofen (both Advil and Motrin tablets) was the primary model compound used throughout systematic method development where one parameter was varied at a time.

Once developed, additional BCS class II drugs were tested for confirmatory purposes. Additionally, the *in vitro* dissolution method was used in various applications including Amgen development compounds and other commercially available products.

Five case studies are presented to demonstrate the potential applications of this *in vitro* biorelevant dissolution method:

I. Rank ordering of development formulations
II. Effect of pH modifier
III. Assessment and prediction of food effect
IV. Dose proportion
V. Lot-to-lot variability

Methods

The *in vitro* biorelevant method described previously in Chapter 2 was used directly where online UV was collected at a product-specific wavelength. See Table 1 for the conditions of the *in vitro* biorelevant dissolution method developed. See Figure 1 for the *in vitro* dissolution results of the model compound, ibuprofen using the final conditions described in Table 1.

Table 1. Biorelevant Dissolution Method Summary

Parameter	**Final Method**
Flow rate	8 mL/min
Size of cell	22.6 mm inner diameter
Sample holder	Presence
Biorelevant medium	Various as needed: • Simulated gastric fluid (SGF) • Simulated intestinal fluid (SIF) • Fasted state simulated intestinal fluid (FaSSIF) • Fed state simulated intestinal fluid (FeSSIF)
Enzyme	Absence
Glass beads	1 mm
Filter pore size	0.7 μm

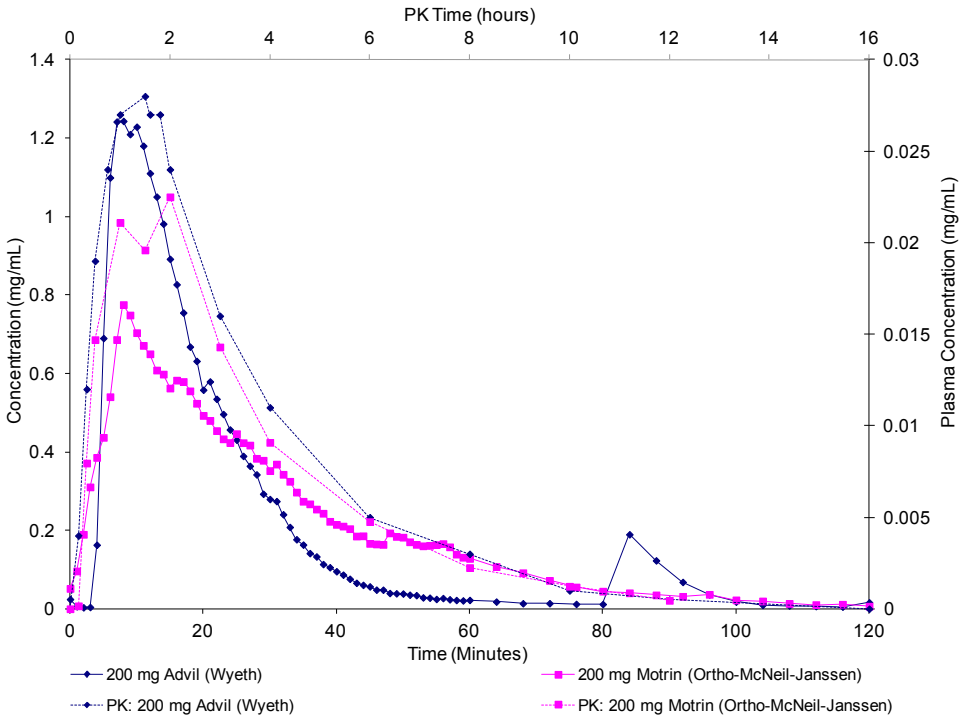

Figure 1. Plasma Profiles and *In Vitro* Dissolution Profiles of 200 mg Advil® and Motrin® at 8 mL/min using 22.6 mm Inner Diameter Flow-Through Cell in SIF (120 minutes)

See Table 2 for the compounds used for each case study.

Table 2. Summary Details Compounds Used in Case Studies

Case Studies	Active Ingredient	Acidic /Basic /Neutral	pKa(s)	Aqueous Solubility (μg/mL)	Strength & Brand Name (if applicable)	Manufacturer
I, III, IV	AMG 853	Weak acid	4.00, 7.90	32	20, 25, 50, 100 mg	Amgen
II	Compound A	Weak base	5.1	0.13	25, 100 mg	Amgen
II, III	Lansoprazole	Basic	17.3	0.97	15, 30 mg Prevacid SoluTabs	TAP
III	Danazol	Neutral	-	0.0176	200 mg danazol	Barr
V	AMG 221	Weak base	1.5	60 (pH 6.1)	10 mg	Amgen

Case Study I. Rank Ordering of Development Formulations

The ability to quickly and concisely select formulations for further clinical development is paramount to drug development. With the use of the *in vitro* biorelevant dissolution method described in this thesis, rank ordering of various development formulations may be evaluated, where AMG 853 is presented in this case study.

AMG 853 is a free acid, small molecule Amgen clinical development candidate that posed significant challenges throughout formulation development because of its low aqueous solubility (32 μg/mL) and high predicted dose. AMG 853 is a BCS Class II compound that is a weak acid with pKa values of 4.0 and 7.9 and a measured logP of 4.5. See Figure 2 for compound's structure.

Two different 20 mg immediate release formulations were initially developed for early clinical development. Each formulation had similar excipients with these noted differences: different grades of microcrystalline cellulose and lactose monohydrate as well as absence or presence of HPMC. See Table 2 for complete formulation composition of each formulation.

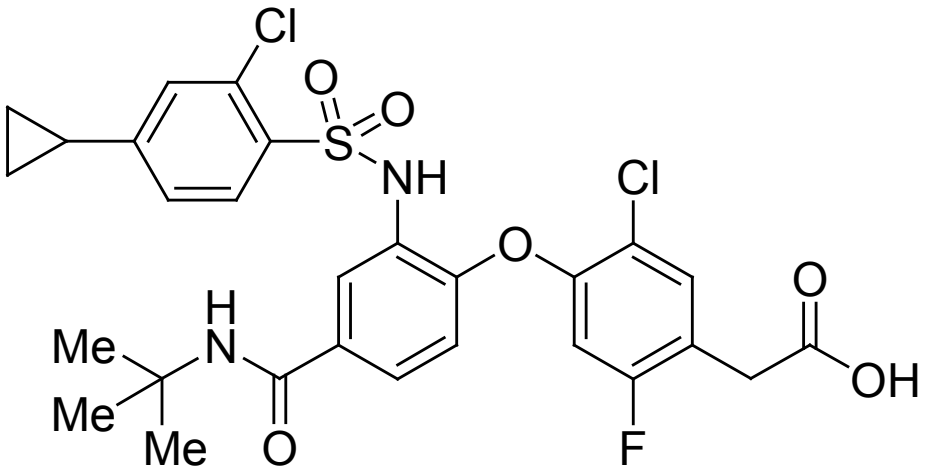

Figure 2. AMG 853 Free Acid Structure

Table 2. Formulation Compositions for AMG 853 Development Lots

		Formulation 1	Formulation 2
	Process:	**Wet Granulation**	**Direct Compression**
	Dosage Form:	**Tablet**	**Tablet**
Ingredient	**Purpose**	**% w/w**	**% w/w**
AMG 853	Active	20.0	20.0
Microcrystalline cellulose, Avicel PH101	Diluent	25.5	-
Microcrystalline cellulose, Avicel PH102	Diluent	-	28.5
Lactose monohydrate, Impalpable 313	Diluent	45.0	-
Lactose monohydrate, Impalpable 316	Diluent	-	45.0
Hypromellose, HPMC-K3 Prem LV	Binder	3.0	-
Sodium Starch Glycolate, Explotab	Disintegrant	4.0	4.0
Poloxamer, Lutrol Micro 68 MP	Surfactant	2.0	2.0
Magnesium Stearate (non-bovine)	Lubricant	0.5	0.5

Forced degradation studies as well as quality control dissolution results using USP apparatus 2 were very similar for the two formulations. However, USP apparatus 4 dissolution results in SIF indicated that the wet granulation formulation (Formulation 1) would have a much better *in vivo* performance versus the direct compression formulation (Formulation 2). See Figures 3 and 4 for concentration and cumulative percent dissolved versus time plots.

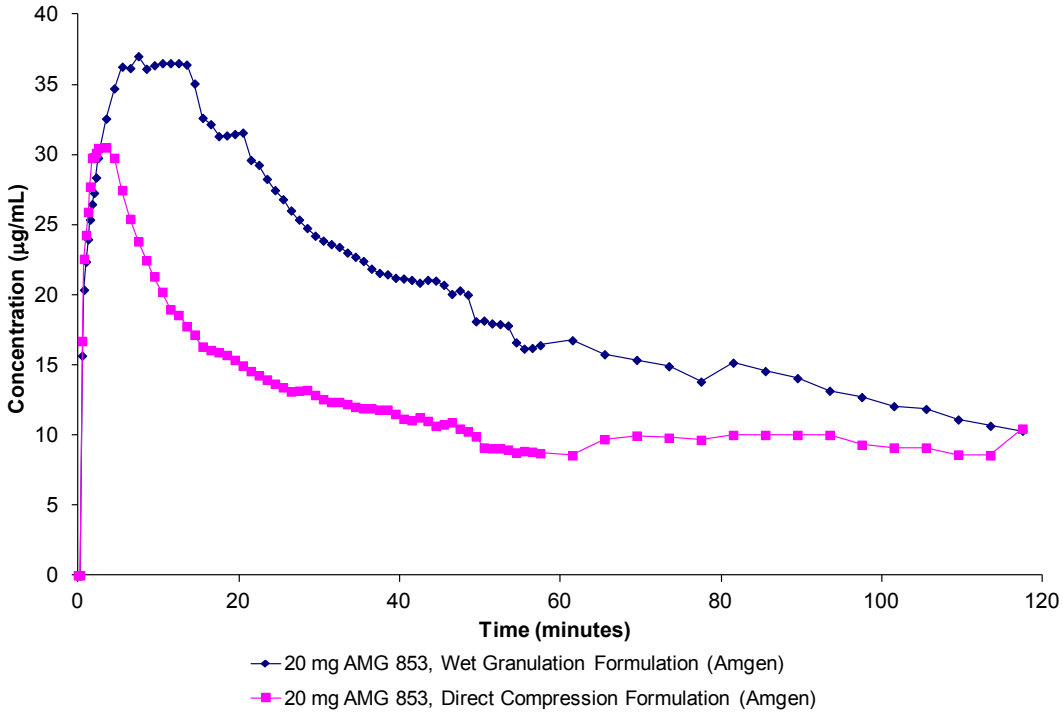

Figure 3. *In Vitro* Concentration Profiles of 20 mg AMG 853 Tablets in SIF

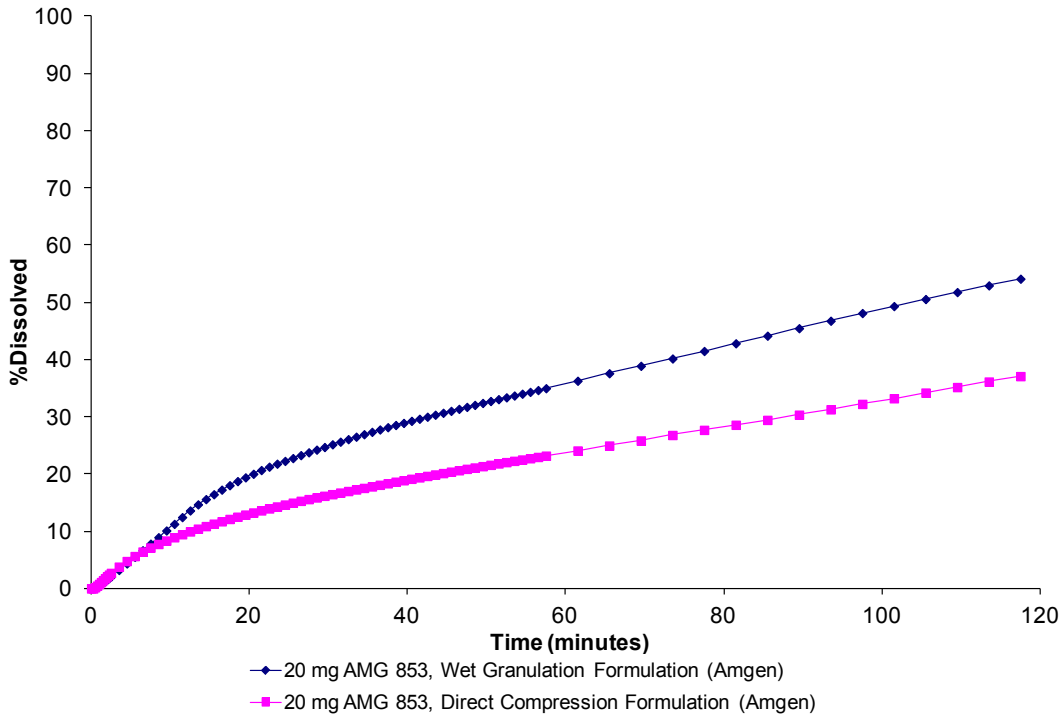

Figure 4. *In Vitro* Percent Dissolved Profiles for 20 mg AMG 853 Tablets in SIF

This prediction was later confirmed by an *in vivo* nonclinical pharmacokinetic (PK) study (cyno monkeys, n = 4), which indicated that although the t_{max} values for Formulation 1 and Formulation 2 were similar, Formulation 1 had approximately three times the C_{max} and approximately four times the AUC compared to Formulation 2. See Figure 5 for PK data. Based on these results, Formulation 1 was selected for further development.

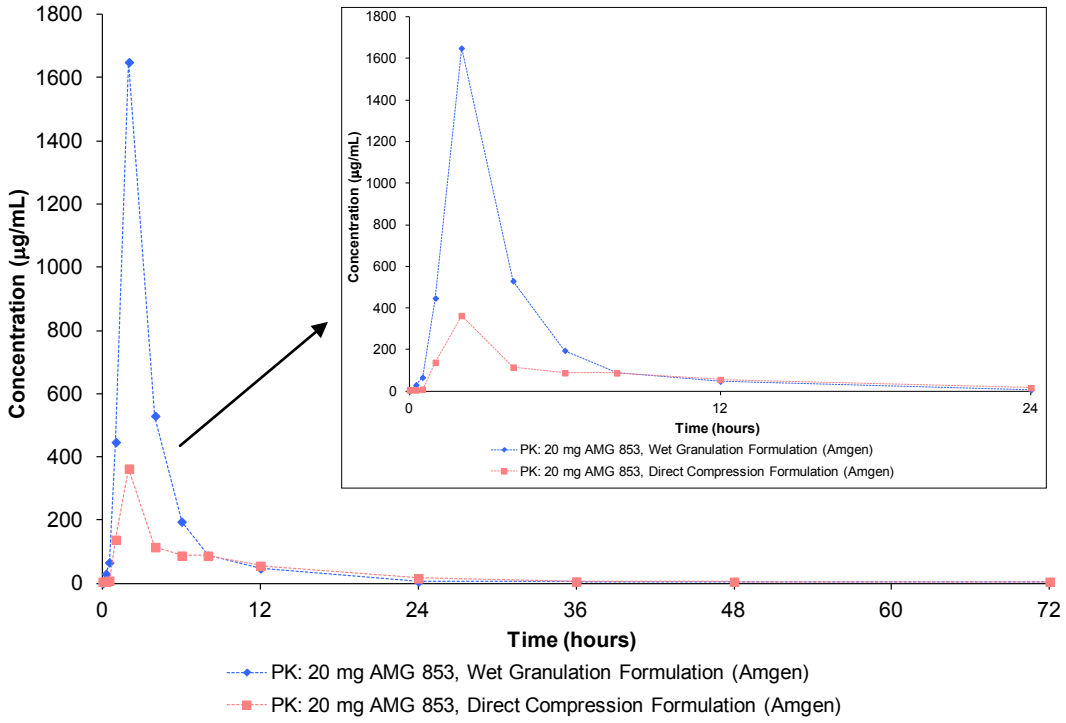

Figure 5. Animal Plasma Profiles for 20 mg AMG 853 Tablets in SIF

Discussion: Case Study I

Oftentimes, *in vitro* dissolution is one of the critical quality attributes that is used to evaluate formulations and subsequently select one formulation from another for further clinical development. However, this objective can be particularly challenging when using traditional dissolution testing, which is often developed with one or few lots of material and often aims to

achieve 100% drug release, none of which may be biorelevant. Based on these limitations of traditional dissolution, the generic *in vitro* biorelevant dissolution method described in this thesis may provide an alternative approach during early phase formulation development, particularly when an *in vitro/in vivo* correlation has not been previously established as is the case for Case Study I.

In this case study, AMG 853 Formulation 1 had significantly better exposure compared to that of Formulation 2, which was subsequently confirmed by monkey PK studies. Based on the data, this may be attributed to the presence of HPMC in Formulation 1, which can significantly improve the wettability of the compound and therefore enhance AMG 853 dissolution.

Case Study II. Effect of pH Modifier

Various formulation techniques are employed to improve a drug's absorption *in vivo*. Such techniques may involve change the microenvironment of the drug with the use of pH modifiers, which can enhance drug solubility at the microenvironment level and may in turn improve the drug's absorption *in vivo*. To that end, Compound A is presented in this case study using the effect of pH modifier to change Compound A's microenvironment in an attempt to improve its low aqueous solubility *in vivo*.

Compound A is an Amgen clinical development compound that is a BCS Class II compound. It is a weak base formulated as a mesylate salt with low aqueous solubility (0.13 μg/mL in pH 6.8 phosphate buffer), a pKa of 5.1 and a log P of 3.3. Compound A was formulated as 25 mg and 100 mg immediate release tablets for early phase clinical studies.

In order to maintain a supersaturated microenvironment at high pH, fumaric acid was used as a pH modifier in two prototype formulations (Lots 2 and 3). Additionally, two formulations without the pH modifying agent, fumaric acid (Lots 1, 4, and 5) were developed to serve as negative controls. See Table 3 for the formulation compositions of Lots 1, 2, 3, 4, and 5.

Lot 2 contains 15% fumaric acid (15% intragranular, 0% extragranular) while Lot 3 contains 20% of fumaric acid (15% intragranular, 5% extragranular). The excipient HPMC-K3 was present in all formulations to minimize precipitation and help maintain supersaturation.

Table 3. Formulation Compositions for Compound A Development Lots

Lot:	**1**	**2**	**3**	**4**	**5**
Process:	**Blend in Capsule**	**Direct Compression**	**Dry Granulation**	**Dry Granulation**	**Dry Granulation**
Dosage Form:	**Capsule**	**Tablet**	**Tablet**	**Tablet**	**Tablet**
Ingredient	**% w/w**	**% w/w**	**% w/w**	**% w/w**	**% w/w**
Compound A Mesylate salt	40.70	40.70	40.70	40.70	39.50
Microcrystalline cellulose, Avicel PH102	14.10	24.20	12.80	15.00	-
Microcrystalline cellulose, Avicel PH200	-	-	-	-	50.00
Lactose monohydrate, Impalpable 316	36.70	11.10	-	24.80	-
Fumaric Acid	-	15.00	20.00	-	-
Hypromellose, HPMC-K3 LV	8.00	4.00	4.00	4.00	4.00
Crospovidone	-	3.00	3.00	4.00	5.00
Syloid 244 FP	-	1.00	1.00	0.25	-
Magnesium Stearate (non-bovine)	0.50	1.00	0.50	0.50	0.75
	-	-	**Extra-granular**		
Syloid 244 FP-ex	-	-	-	0.25	-
Fumaric Acid-ex	-	-	5.00	-	-
Microcrystalline cellulose, Avicel PH102-ex	-	-	12.50	10.00	-
Magnesium Stearate-ex (non-bovine)	-	-	0.50	0.50	0.75

The biorelevant dissolution results using SGF (5 minutes) followed by SIF (120 minutes) predicted that Lots 1, 2, 3, and 4 would have similar *in vivo* bioavailability. Additionally, results

indicated that the formulations with pH modifiers (Lots -2 and 3) would have similar *in vivo* performance compared to the lots without pH modifiers (Lots 1 and 4) all exhibiting similar cumulative percent dissolved (~20%). See Figures 6 and 7 for the biorelevant dissolution data.

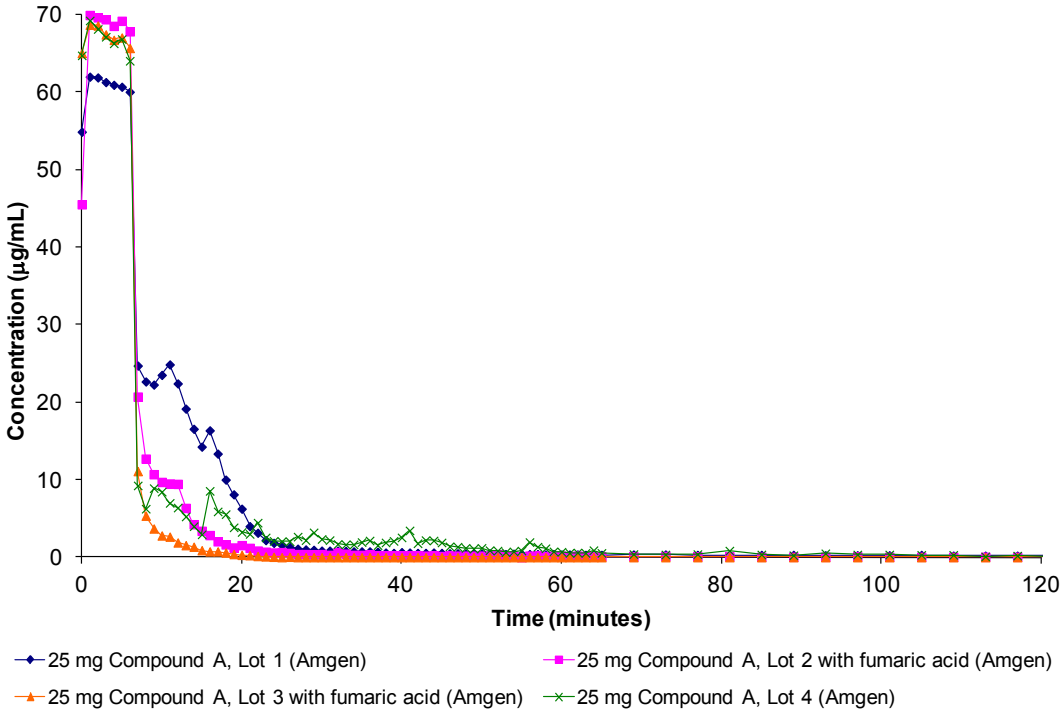

Figure 6. *In Vitro* Concentration Profiles of 25 mg Compound A Tablets in SGF → SIF

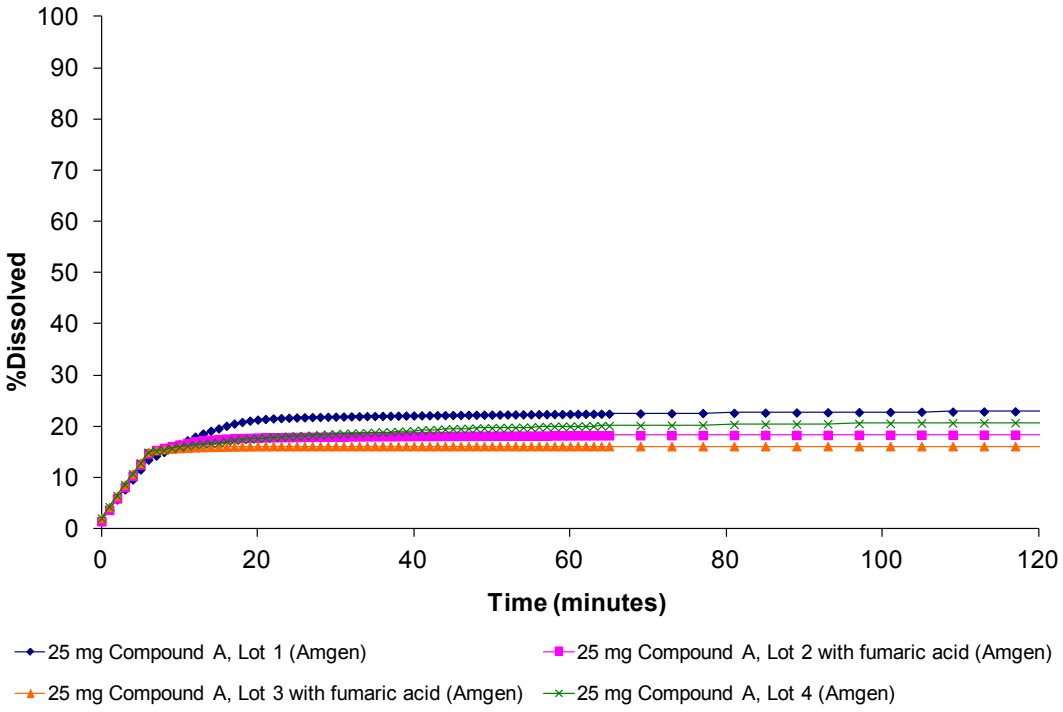

Figure 7. *In Vitro* Percent Dissolved Profiles for 25 mg Compound A Tablets in SGF → SIF

Several pharmacokinetic studies (male beagle dogs, n = 5) indicated no significant difference in maximum concentration (C_{max}) and area under the curve (AUC) for the formulations tested, which conforms well with the *in vitro* data. See Figure 8 for the animal plasma profiles.

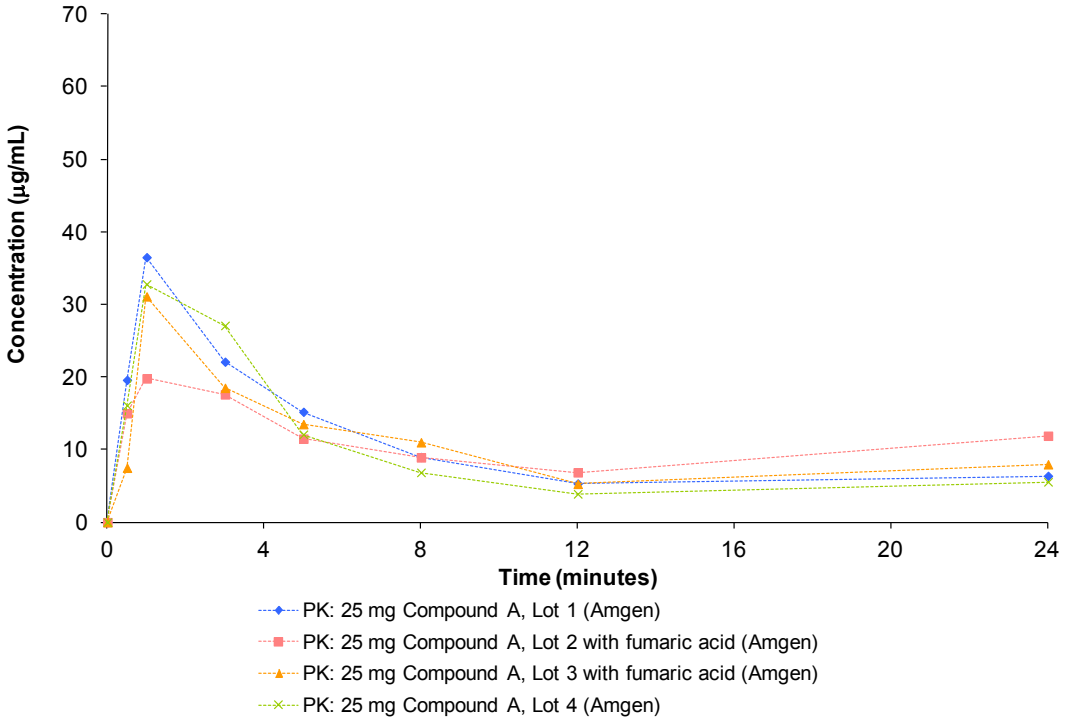

Figure 8. Animal Plasma Profiles for 25 mg Compound A Tablets

However, when the experiments were conducted in SIF directly, the data showed a noticeable difference between the formulations with and without pH modifier. See Figures 9 and 10 for the concentration and cumulative percent dissolved versus time biorelevant dissolution data, respectively. Note that this experiment did not include Lot 1 due to limited supplies, but rather included an additional tablet Lot 5. Please refer to Table 3 for the corresponding formulation composition for Lot 5. As the figures indicate, Lots 2 and 3 exhibited significantly higher dissolution compared to Lots 4 and 5.

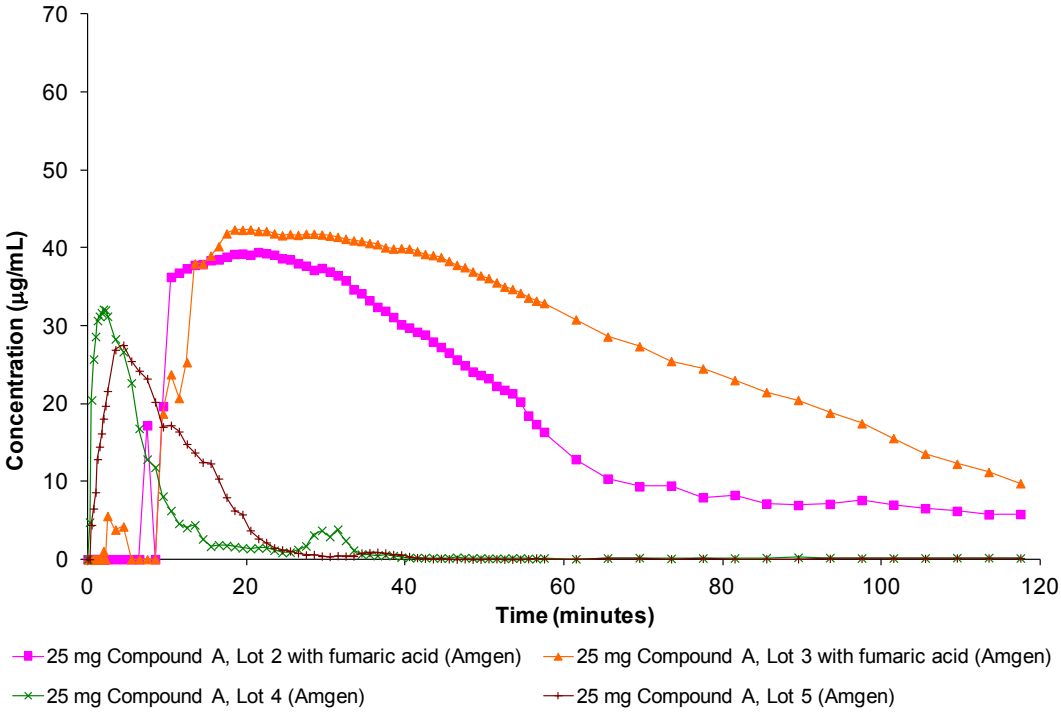

Figure 9. *In Vitro* Concentration Profiles of 25 mg Compound A Tablets in SIF

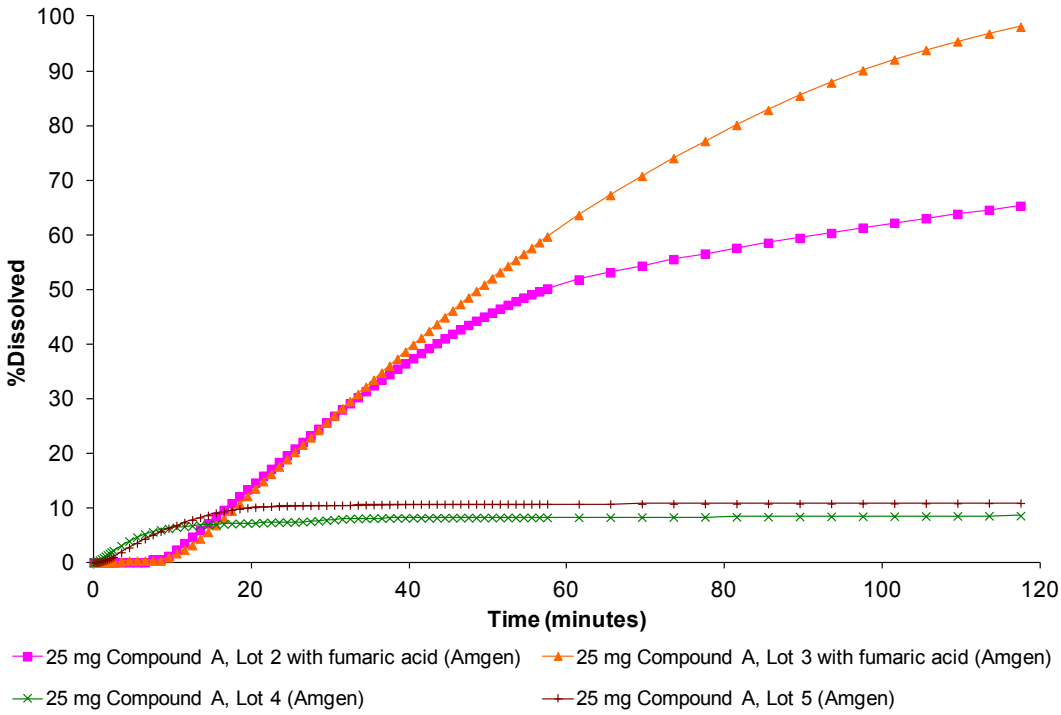

Figure 10. *In Vitro* Percent Dissolved Profiles of 25 mg Compound A Tablets in SIF

As noted earlier, Lots 2 and 3 both included an acid modifier (i.e., fumaric acid), which was a formulation technique to maintain an acidic microenvironment during absorption and therefore enhance *in vivo* dissolution. The results indicated that when the drug was released in a higher pH environment (neutral or alkaline pH), the use of a weak acid modifier is a good strategy to enhance drug bioavailability. However, if the drug disintegrates and releases in a lower pH environment (acidic stomach), the weak fumaric acid may not function effectively as an internal pH modifier and may account for the lack of discrimination between formulations seen *in vivo*.

For comparison purposes, 15 mg Prevacid SoluTabs were tested using the same *in vitro* biorelevant dissolution method using SIF as the dissolution medium. Prevacid SoluTabs are commonly used as a proton pump inhibitor to treat various acid-related disorders, where the active ingredient is lansoprazole, a BCS class II compound that is a weak base with a pKa value of 4.15. See Figure 11 for the molecular structure of lansoprazole.

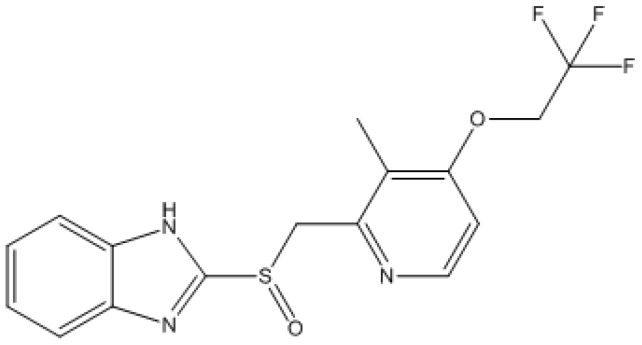

Figure 11. Lansoprazole molecular structure

In contrast to the Compound A tablets, the Prevacid tablets are enteric-coated tablets and contain two weak acid pH modifiers, methacrylic acid and citric acid. (1) The observations in

the *in vitro* biorelevant dissolution studies using 15 mg Prevacid SoluTabs were very different from those for Compound A (Figure 12). This may be attributed to the fact that the microgranules that comprise Prevacid tablets are enteric-coated. Because of this enteric coating, the drug can only be released in the neutral or alkaline environment (i.e., duodenum). As a result, the two weak acids present in this tablet formulation functioned as intended, as pH modifiers in this microenvironment.

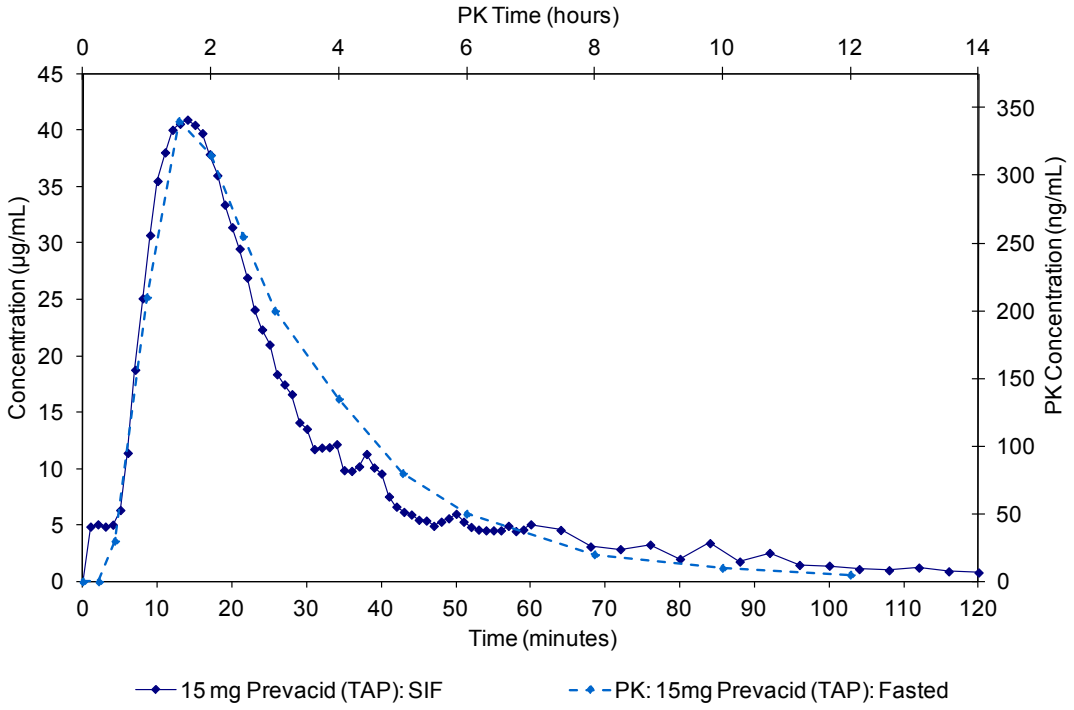

Figure 12. Plasma Profile and *In Vitro* Concentration Profile of 15 mg Prevacid Tablets

Discussion: Case Study II

Based on the data presented thus far in this case study, the results from the *in vitro* biorelevant dissolution testing and PK study indicated that there are no significant differences observed for the prototype formulations of Compound A. In particular, there was no advantage seen in the formulations with fumaric acid was used as a pH modifier (Lots 2 and 3) versus the

lots without pH modifier (Lots 1 and 4). This may be due to the fact that Compound A, a mesylate salt of weak base that was formulated as an immediate-release tablet with fumaric acid used as a pH modifier, where the table disintegrated and was released in the stomach where strong acidic conditions are commonly observed. In the strongly acidic environment of the stomach, it is conceivable that the designed microenvironment that was expected to be modified by the weak acid was difficult to establish.

In contrast, the effect of the pH modifiers for the Prevacid SoluTabs® was clearly observed. In this case, the tablets were formulated as delayed-release orally disintegrating tablets with the use of enteric-coated microgranules. Although the tablets were designed to disintegrate quickly in the mouth, the enteric-coated microgranules allowed the drug to travel through the upper GIT unscathed, which was later to be released in the neutral or alkaline environment of the duodenum. As a result, the weak acids (i.e., methacrylic acid and citric acid) in the Prevacid SoluTab formulation were able to function effectively as pH modifiers in the neutral or alkaline environment of the duodenum.

Additionally, the presence of enteric-coated microgranules may also explain why similar dissolution profiles are observed regardless of a medium switch (i.e., SIF data versus SGF – SIF data), which is very different from the case study for Compound A. See Figure 13 for the biorelevant dissolution data with and without the medium switch.

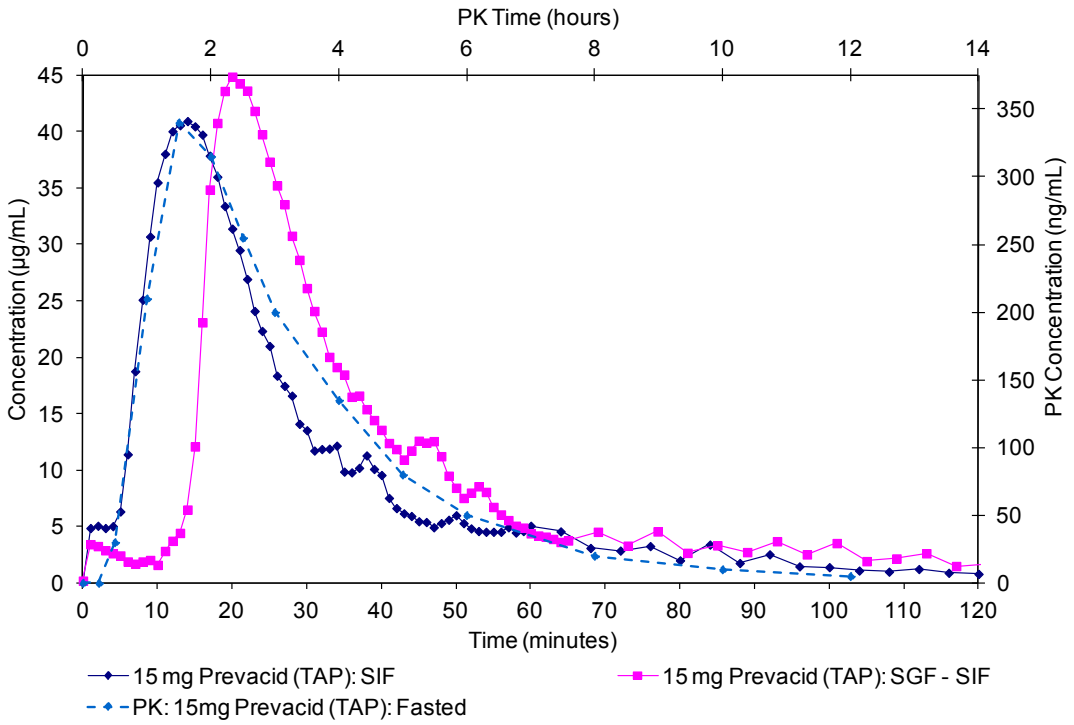

Figure 13. Plasma Profile and *In Vitro* Concentration Profiles of 15 mg Prevacid Tablets

The differing observations made for Compound A and Prevacid SoluTabs® indicate that the use of a delayed-release strategy (e.g., enteric-coated microgranules) may significantly enhance the effectiveness of a weak acid as a pH modifier in a dosage form such was the case for the Prevacid SoluTabs®. Otherwise, without the use of enteric-coated microgranules, as the dosage form disintegrates and the drug is released into the stomach at low pH, the weak acid in the dosage form may be "washed away" and therefore the desired effect of the pH modifier may not be seen.

Case Study III. Assessment and Prediction of Food Effect

The effect of food on drug absorption and hence on the bioavailability of small molecule drugs has been shown to be one of the critical elements that impacts successful drug

development (*2, 3, 4*). With this in mind, there is a great need to understand and predict food effects early in development to maximize overall drug bioavailability and help design the most effective animal and human PK studies. With the use of FaSSIF and FeSSIF, food effect may be qualitatively evaluated using the *in vitro* USP apparatus 4 biorelevant dissolution method.

Numerous compounds have been tested using this approach to assess or confirm different drug release rates under fasted or fed conditions (*5*). Lansoprazole (15 mg Prevacid SoluTabs®), danazol (200 mg danazol capsules), and Amgen development compound AMG 853 were evaluated for food effect using USP apparatus 4.

See Figures 14 and 15 for *in vitro* dissolution results for lansoprazole and danazol, respectively.

As previously described in Case Study I, AMG 853 is a free acid, small molecule Amgen clinical development candidate that posed significant challenges throughout formulation development because of its low aqueous solubility (32 μg/mL) and high predicted dose. AMG 853 is a BCS Class II compound that is a weak acid with pKa values of 4.0 and 7.9 and a measured logP of 4.5. AMG 853 was formulated as immediate-release 25 mg and 100 mg tablets for the clinic. See Figure 2 for compound's structure and Figures 16 and 17 for the *in vitro* dissolution results for AMG 853, including concentration and cumulative percent dissolved versus time, respectively. Figure 18 describes the AMG 853 pharmacokinetic data.

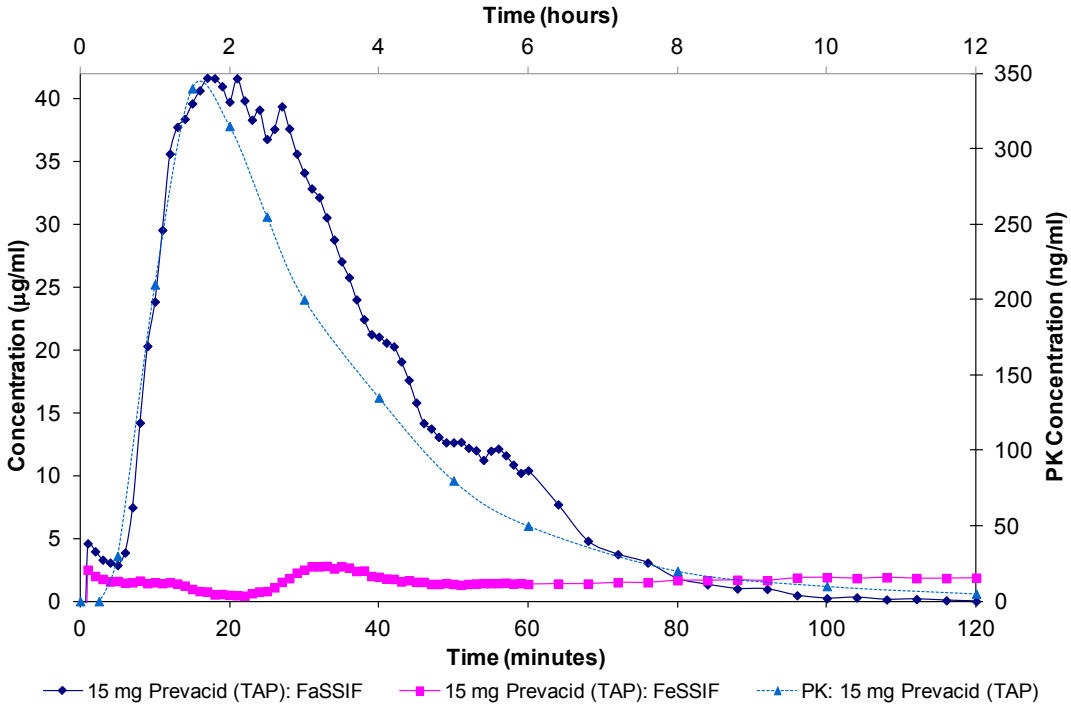

Figure 14. Plasma Profile and *In Vitro* Concentration Profile of 15 mg Prevacid Tablets

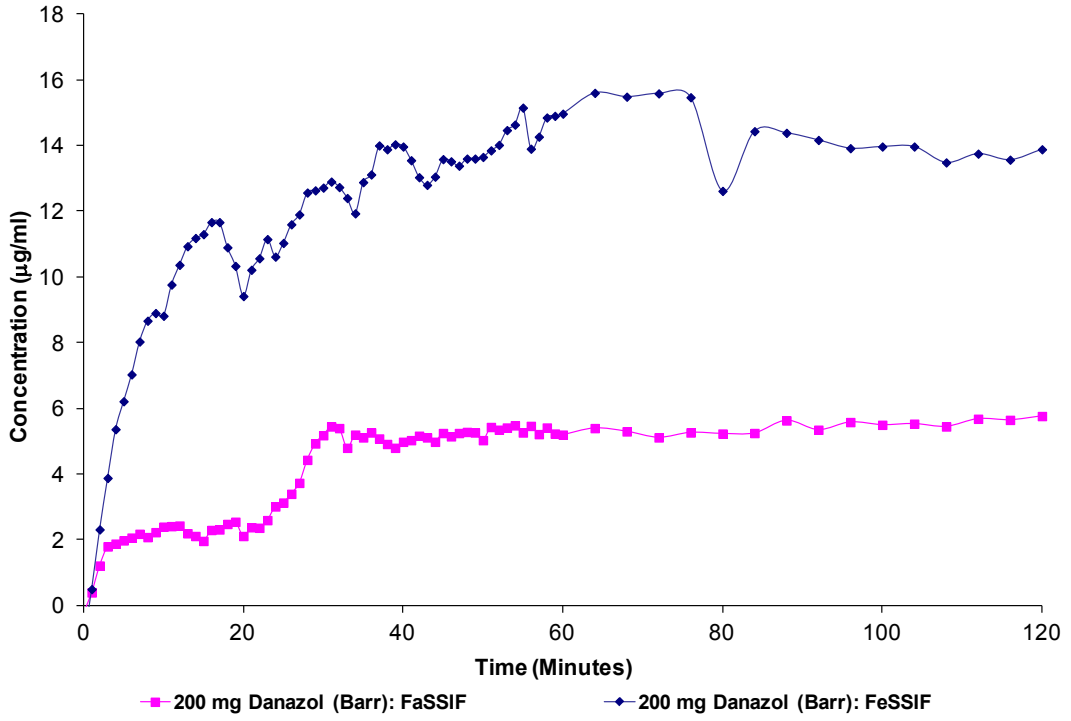

Figure 15. *In Vitro* Concentration Profile of 200 mg Danazol Capsules in FaSSIF and FeSSIF

Lansoprazole should be administered under fasted conditions (*6, 7*). In agreement with this recommendation, the *in vitro* biorelevant dissolution test results indicate that lansoprazole would have significantly higher bioavailability in the fasted state versus the fed state (Figure 14). It was reported that danazol has at least three times higher bioavailability in the fed state was observed versus the fasted state (*8, 9*), which was confirmed by the *in vitro* biorelevant dissolut7ion data (Figure 15).

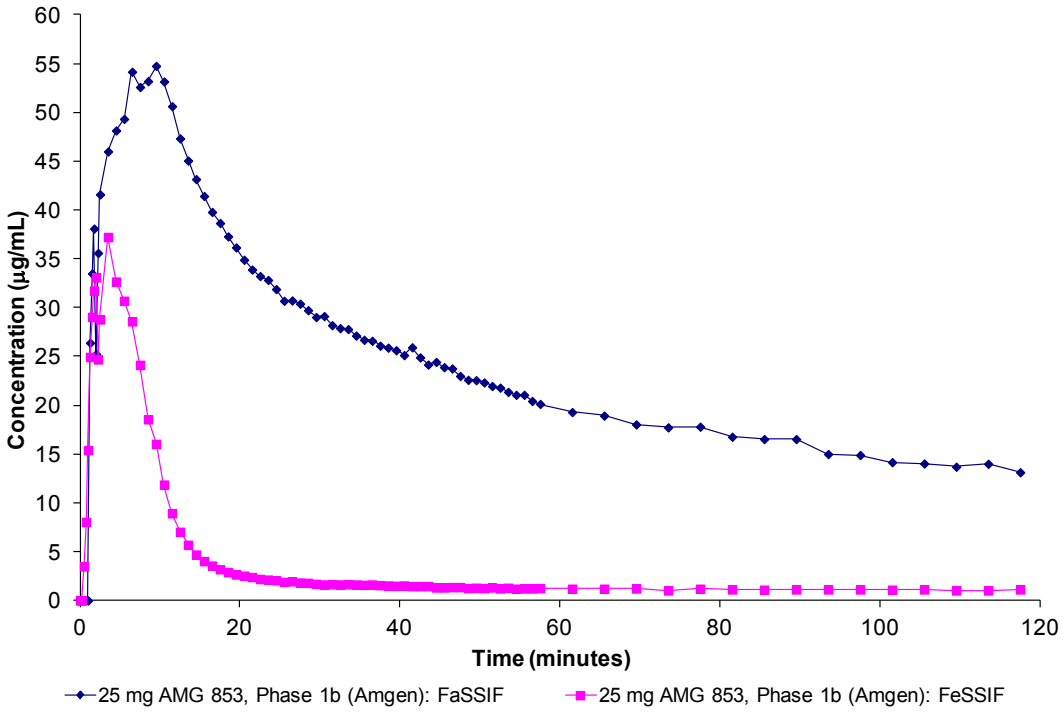

Figure 16. *In Vitro* Concentration Profile of 25 mg AMG 853 Phase 1b Tablets in FaSSIF and FeSSIF

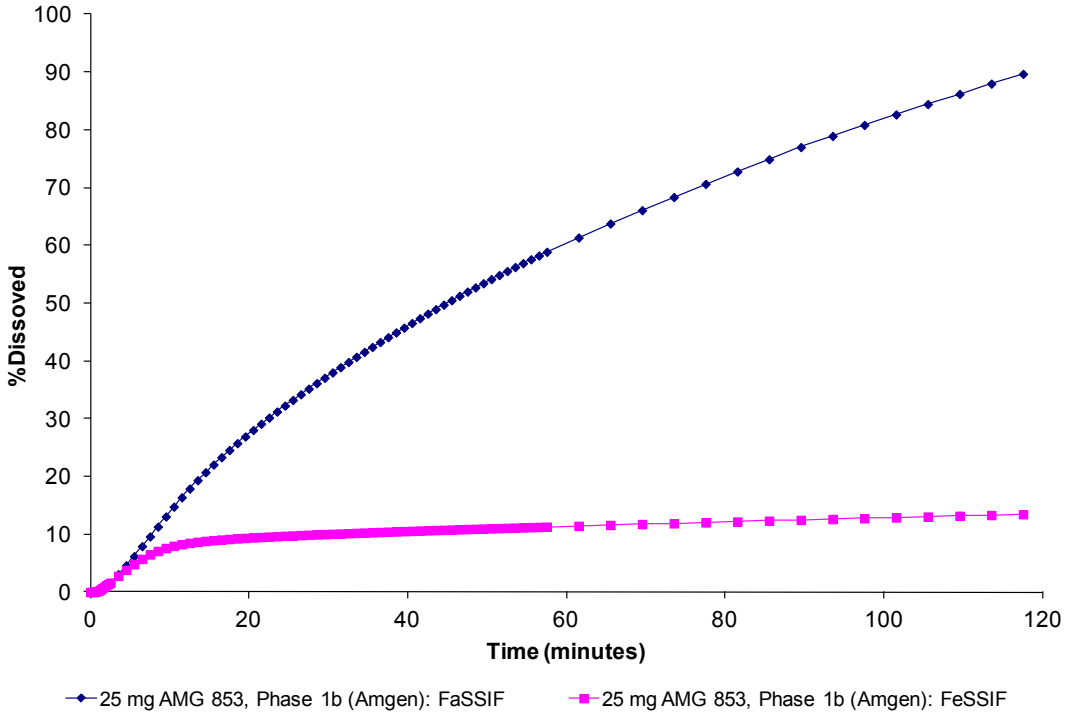

Figure 17. Percent Dissolved Dissolution Data for 25 mg AMG 853 Phase 1b Tablets in FaSSIF and FeSSIF

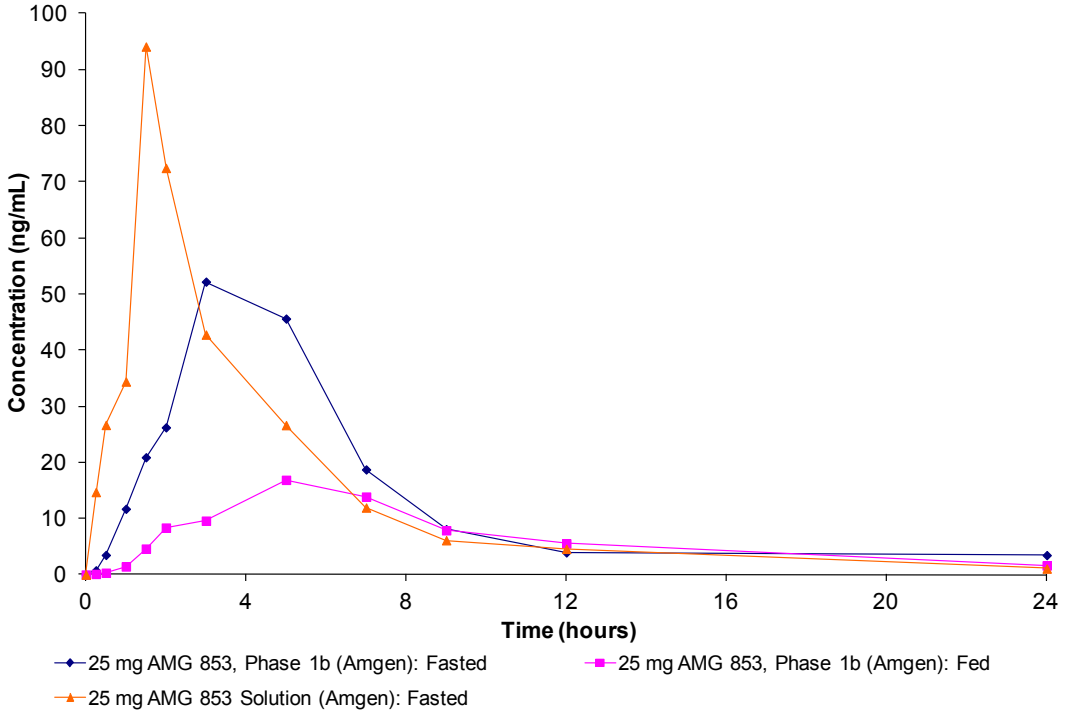

Figure 18. Animal Plasma Profiles for AMG 853

In vitro biorelevant dissolution results for the Phase 1b AMG 853 tablets using USP apparatus 4 with both FaSSIF and FeSSIF suggest that this product has a significant negative food effect with higher bioavailability seen in the fasted condition compared to the fed condition (Figures 16 and 17). The results of the Phase 1b tablets were later confirmed with a partial-crossover human PK study (n = 6) where C_{max} values of 58.3 and 18.6 ng/mL and AUC values of 335 and 114 h.ng/mL for the fasted and fed states, respectively were observed (Figure 19). Note that the solution and tablet dosed in the fasted condition have the same AUC, whereas the tablet dosed in the fed condition results in an approximate 50% decrease in AUC. Additionally, dose proportion was not observed at higher strengths. At the time of this clinical study, no suitable salts, hydrates, or other potential forms were identified.

Based on the negative food effect observed, several alternate formulations were evaluated to mitigate this food effect risk during Phase 2a formulation development. These formulations include a sodium salt formulation as well as several amorphous formulations including hot melt extrusion, solid dispersion, and lipid-filled capsules. Of these formulations, two were available for *in vitro* biorelevant dissolution testing (sodium salt and hot melt extrusion) and were compared to the original clinical trial material. See Tables 4 and 5 for the formulation compositions of all AMG 853 tablets described in this case study. See Figures 19, 20, 21, and 22 for respective dissolution data. Please note that the *in vitro* dissolution was conducted in SGF (5 minutes) followed by FaSSIF (120 minutes) to mimic the worst-case scenario and simulate the GIT observed under this negative food effect.

Table 4. Formulation Compositions for 25 mg AMG 853 Tablet Formulations

Lot:	**0010011635 (Phase 1b)**	**0010026598 (Phase 2a)**
Process:	**Wet Granulation**	**Wet Granulation**
Ingredient	**% w/w**	**% w/w**
AMG 853	5.0	5.0
Lactose monohydrate, Impalpable 313	56.0	56.0
Microcrystalline cellulose, Avicel PH102	29.0	29.0
Sodium starch glycolate, Explotab	4.0	4.0
Hypromellose, HPMC-K3 Prem LV	3.0	3.0
Poloxmer, Lutrol Micro 68 MP	2.0	2.0
Magnesium stearate (non-bovine)	1.0	1.0
Purified water, Granulating fluid	-*	-*
Spray rate, Granulating fluid	5%/min	7.5%/min
Granulation fluid level, Granulating fluid	37.7%	34.9%

*Removed from process during drying

Table 5. Formulation Compositions for 25 mg AMG 853 Alternate Tablet Formulations

Description:	**Na Salt, Lot 1**	**Na Salt, Lot 2**	**Hot Melt Extrusion, Lot 1**	**Hot Melt Extrusion, Lot 2**
Process:	**Direct Compression with Na Salt***	**Direct Compression with Na Salt***	**Hot Melt Extrusion with PVP/PVP-VA[#]**	**Hot Melt Extrusion with PVP^**
Ingredient	**% w/w**	**% w/w**	**% w/w**	**% w/w**
AMG 853	5.2*	5.2*	40.0[#]	40.0^
Lactose monohydrate, Impalpable 313	55.8	54.8	-	-
Lactose monohydrate, Impalpable 316	-	-	20.0	20.0
Microcrystalline cellulose Avicel PH 101	29.0	29.0	-	-
Microcrystalline cellulose, Avicel PH102	-	-	33.0	33.0
Sodium starch glycolate, Explotab	4.0	4.0	6.0	6.0
Hypromellose, HPMC-K3 Prem LV	3.0	3.0	-	-
Poloxmer, Lutrol Micro 68 MP	2.0	2.0	-	-
Sodium carbonate anhydrous	-	1.0	-	-
Magnesium stearate (non-bovine)	1.0	1.0	1.0	1.0

*AMG 853 Na salt
[#]50.6% AMG 853/49.4% Vinylpyrrolidone-vinyl acetate (PVP-VA) 64 (w/w)
^50.6% AMG 853/30.% Vinylpyrrolidone-vinyl acetate (PVP-VA) 64/19.4% Vinylpyrrolidone (PVP) (w/w/w)

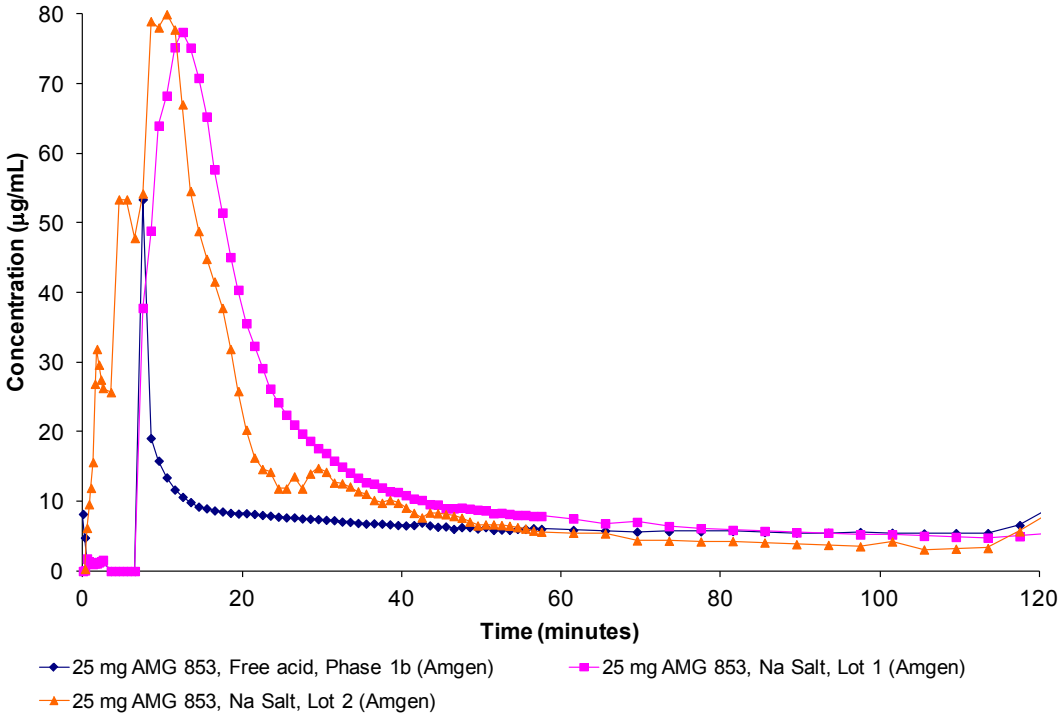

Figure 19. *In Vitro* Concentration Profiles of 25 mg AMG 853 Free Acid (Phase 1b) and Na Salt Tablets

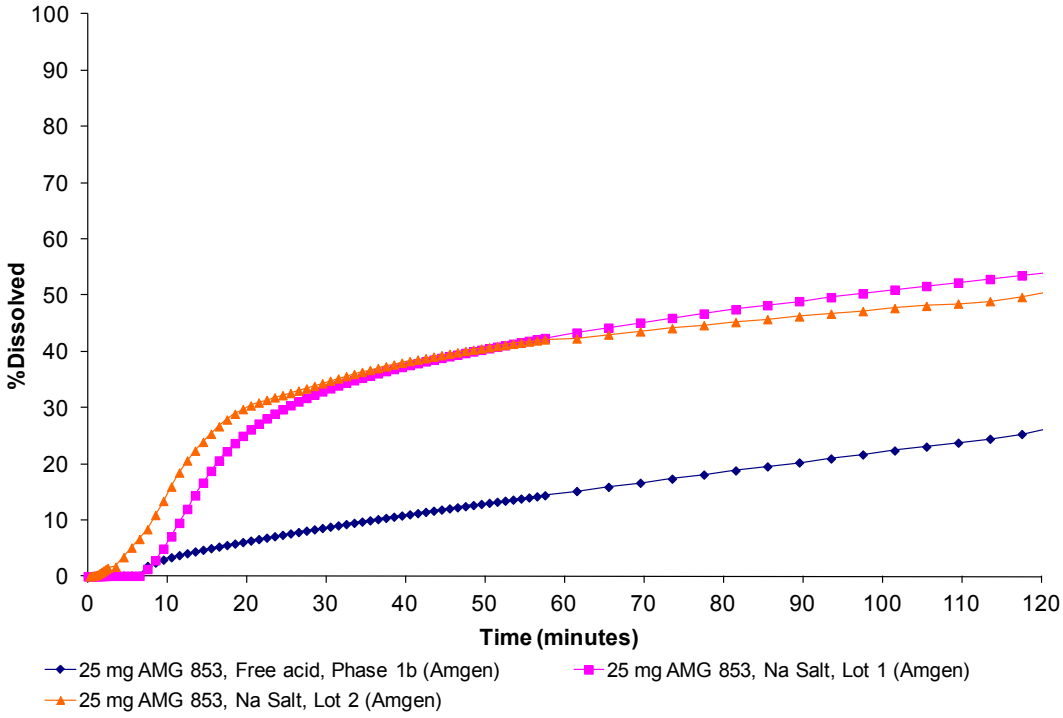

Figure 20. *In Vitro* Percent Dissolved Profiles of 25 mg AMG 853 Free Acid (Phase 1b) and Na Salt Tablets

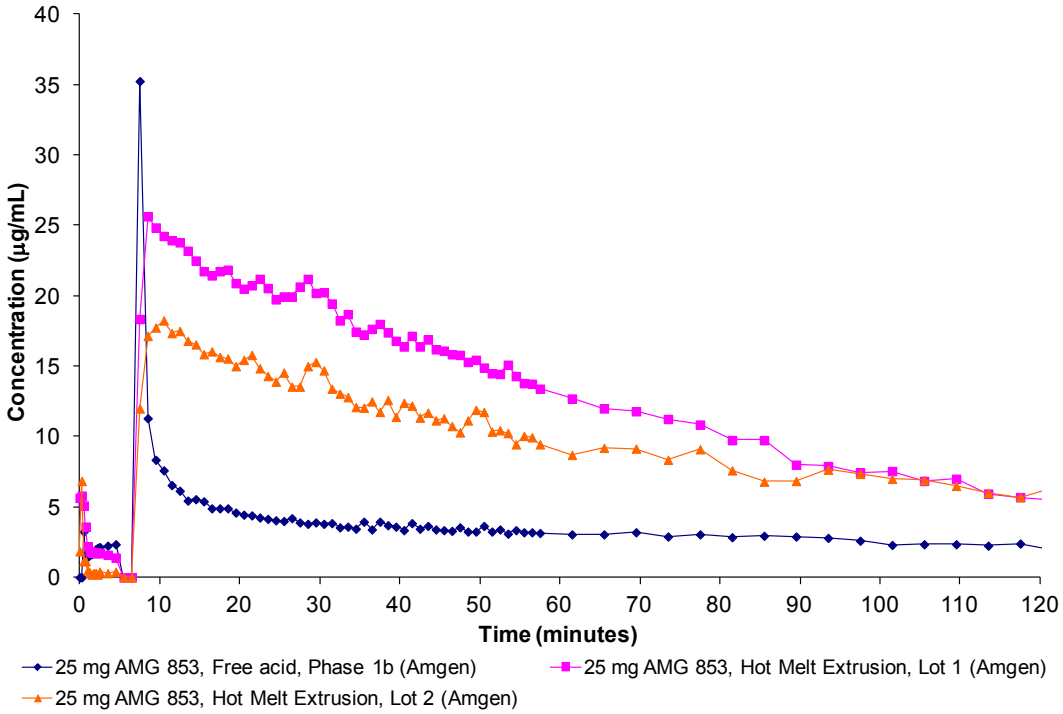

Figure 21. *In Vitro* Concentration Profiles of 25 mg AMG 853 Free Acid (Phase 1b) and Hot Melt Extrusion Tablets

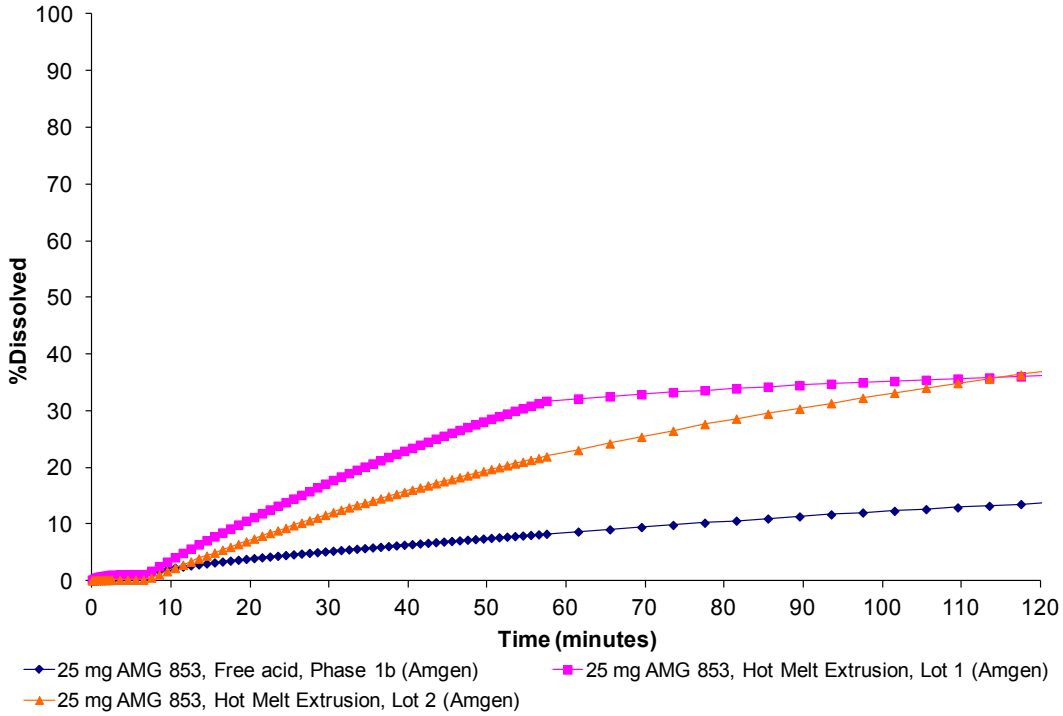

Figure 22. *In Vitro* Percent Dissolved Dissolution Data for 25 mg AMG 853 Free Acid (Phase 1b) and Hot Melt Extrusion Tablets

Discussion: Case Study III

Based on the data presented in case study III, the results suggest that one cause of food effect, or the effect of bile salts may be assessed by *in vitro* means using this biorelevant dissolution method with FaSSIF and FeSSIF. Specifically, danazol, a poorly soluble neutral compound, demonstrated a significant positive food effect in the fed state, similar to troglitzone, a poorly soluble lipophilic weak acid (*10*). This may be explained by the fact that danazol is a lipophilic compound with a logP of 4.2. The increase of bile salt and lecithin concentrations in the fed state may play a key role to enhance the solubility of danazol.

On the other hand, the negative food effect observed for AMG 853, a weak acid with pKa values of 4.0 and 7.9 and a measured logP of 4.5, might be attributed to its pH-dependent solubility, where solubility increases markedly as pH increases.

Case Study IV. Dose Proportion

As previously described in Case Studies I and III, AMG 853 is a free acid, small molecule Amgen clinical development candidate that posed significant challenges throughout formulation development because of its low aqueous solubility (32 μg/mL) and high predicted dose. AMG 853 is a BCS Class II compound that is a weak acid with pKa values of 4.0 and 7.9 and a measured logP of 4.5. See Figure 2 for compound's structure.

Recall from Case Study III that AMG 853 has a significant negative food effect with higher bioavailability seen in the fasted condition compared to the fed condition for both *in vitro* biorelevant dissolution using USP apparatus 4 (Figures 16 and 17) as well as a partial-crossover human PK study (n = 6, Figure 19). Note that the solution and tablet dosed in the fasted

condition have the same AUC, whereas the tablet dosed in the fed condition has an approximate 50% decrease in AUC. Additionally, dose proportion was not observed at higher strengths.

AMG 853 was intended to treat asthma, and therefore at the time it was important to understand any dose proportion issues that might have restricted the dosing regimen of the drug. With this in mind, three doses within the dose range, 5, 25, and 50 mg were evaluated to assess dose proportion using the FaSSIF which gave the greatest bioavailablility for AMG 853 during human PK studies (Figure 18). Note that in the clinic, the 50 mg strength was dosed via two-25 mg tablets. In the same way, this *in vitro* experiment mimicked the same dosing regimen to better understand the implications *in vivo*. See Table 6 for the dosing regimen of 5 and 25 mg AMG 853 tablets covering the range of 5 mg – 200 mg doses. See Figures 23 and 24 for *in vitro* dissolution data, including concentration and cumulative percent dissolved versus time, respectively while Table 7 describes the C_{max} and AUC data for the 5 mg, 25 mg, and 50 mg doses for the *in vitro* dissolution data.

Table 6. Phase 1b Clinical Dosing Regimen for AMG 853 Tablets

Dose (mg)	# of 5 mg Tablets	# of 25 mg Tablets
5	1	-
10	2	-
25	-	1
50	-	2
75	-	3
100	-	4
150	-	6
200	-	8

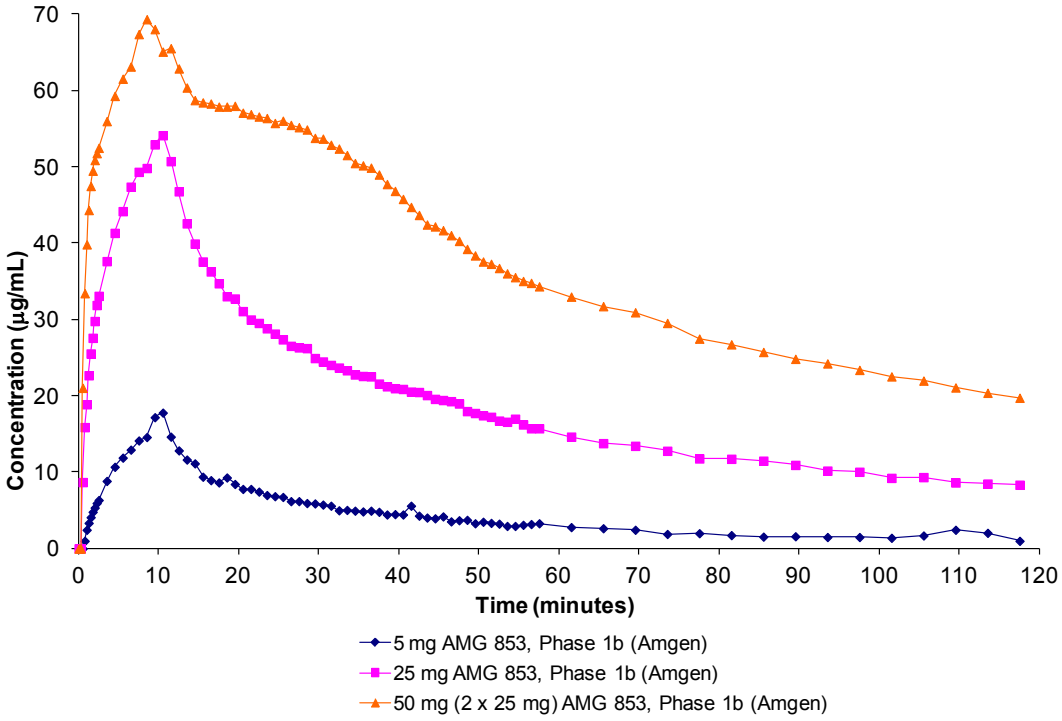

Figure 23. *In Vitro* Concentration Profiles of 5 mg, 25 mg, and 50 mg AMG 853 Tablet Doses

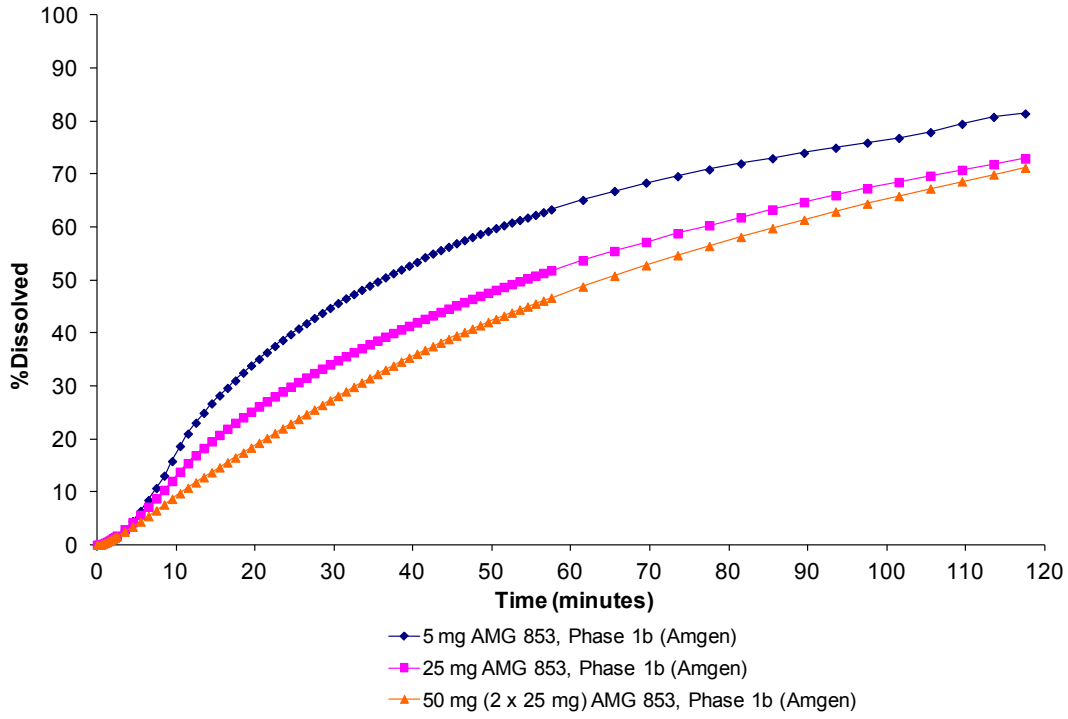

Figure 24. *In Vitro* Percent Dissolved Dissolution Profiles of 5 mg, 25 mg, and 50 mg AMG 853 Tablet Doses

Table 7. C_{max} and AUC Data for 5 mg, 25 mg, and 50 mg AMG 853 Doses

Dose	Cmax (µg/mL)	Versus 5 mg	AUC (mg*min/mL)	Versus 5 mg
5 mg	18	-	0.5	-
25 mg	54	3X	2.3	5X
50 mg (2 x 25 mg)	69	4X	4.5	9X

The 5 mg, 25 mg, and 50 mg doses all have similar concentration versus time profiles (Figure 23). C_{max}, which is a measure of rate of absorption increased with increasing dose with the 50 mg dose giving the largest C_{max} followed by 25 mg and 5 mg doses as expected. The cumulative percent dissolved plot gave an inverse relationship versus C_{max} with the largest cumulative percent dissolved observed in the 5 mg tablet, followed by the 25 mg and 50 mg doses.

Table 7 indicates an increase of approximately 3 times in C_{max} of the 25 mg dose versus the 5 mg dose and approximately 4 times for the 50 mg dose. The table also indicates that AUC, which is a measure of extent of absorption, increased approximately 5 times for the 25 mg dose versus the 5 mg dose and approximately 9 times for the 50 mg dose. Based on the AUC comparison, the data indicates that the 5, 25, and 50 mg doses are approximately proportional. Additionally, the data indicates that AMG 853 dissolves at a similar rate across all doses tested.

Note that testing on the 100 mg dose was not completed due to filter clogging during *in vitro* dissolution testing. In order to test the 100 mg dose, four 25 mg tablets were needed, each individual tablet weighing 600 mg resulting in more than 2 grams of total material within the USP apparatus 4 flow cell. Even with the addition of approximately 0.2 grams of glass wool,

which is added to minimize the amount of excipients/materials that the filter encounters, the filters still tended to over-pressurize and clog compromising the subsequent online UV data collection. Due to these experiment issues, dose proportion for AMG 853 could not be assessed above 50 mg.

Discussion: Case Study IV

The results from case study IV suggest that it may be possible to assess dose proportion for a drug as was seen in for 5 mg, 25 mg, and 50 mg doses of AMG 853. However, it is important to note that such evaluations may be limited by the physical constraints of the USP apparatus 4 flow-through cell. Specifically, doses above 50 mg could not be assessed for AMG 853 because at least four 25 mg tablets are needed, which amounts to over 2 grams of total material. This amount of material, even with the additional of glass wool as an additional filtration step, appears to exceed the limitations of the filter paper and therefore it is believed that this application, evaluation of dose proportion, has limited utility during drug development.

Case Study V. Lot-to-Lot Variability

AMG 221 is classified as a BCS Class II compound according to the Biopharmaceutics Classification System. It is a weak base with a pKa of 1.5. See Figure 25 for its structure. AMG 221 was formulated as a 10 mg immediate-release tablet for early phase clinical studies.

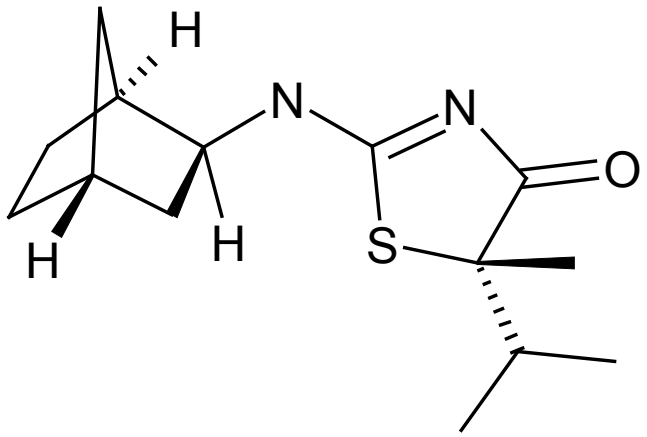

Figure 25. AMG 221 Free Base Structure

During release testing of a re-supply lot slower disintegration and dissolution was observed when using the quality control release method (USP apparatus 2, 900 mL, 0.1N HCl, 50 rpm). See Figures 26 and 27 for the dissolution data.

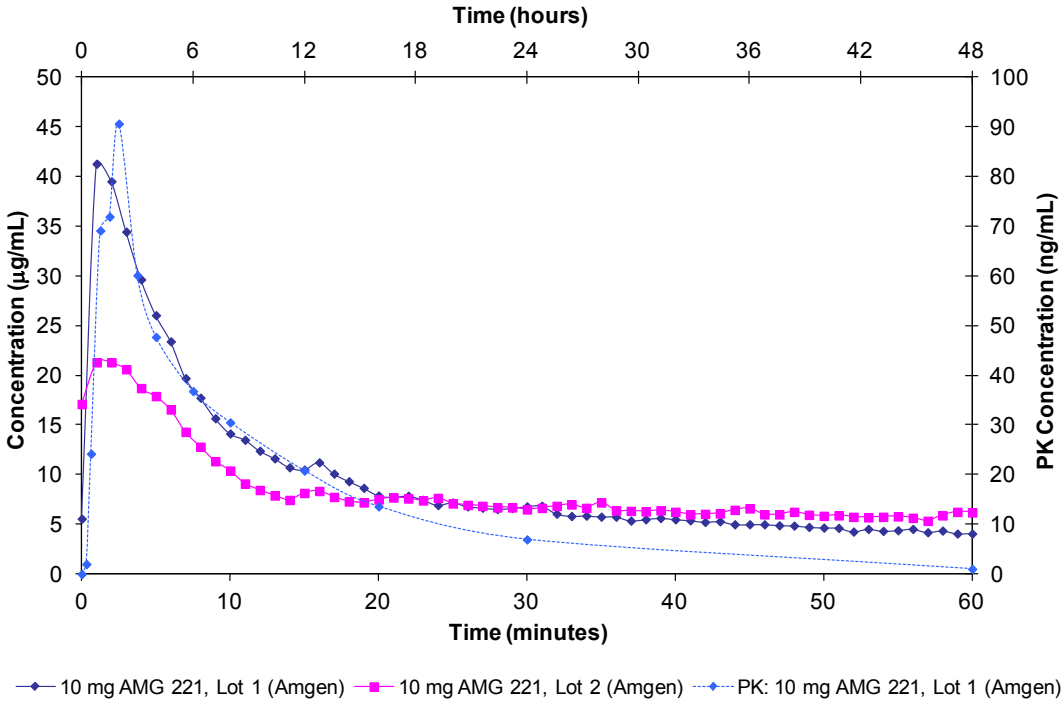

Figure 26. Plasma Profile and *In Vitro* Concentration Profiles for 10 mg AMG 221 Tablets

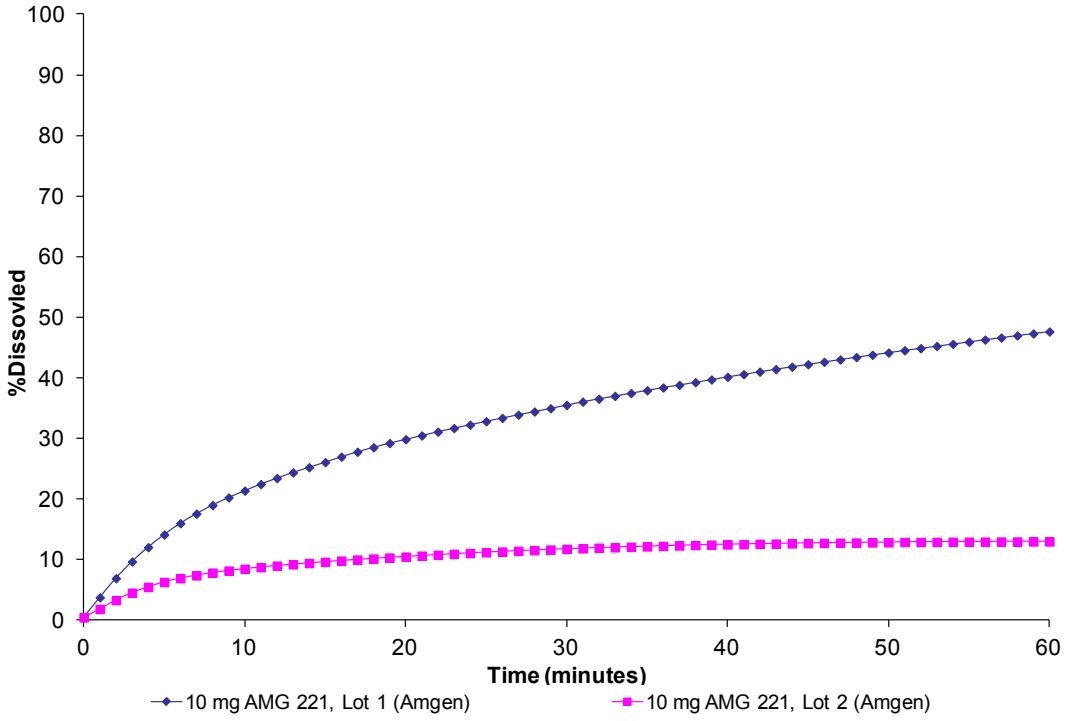

Figure 27. *In Vitro* Percent Dissolved Profiles for 10 mg AMG 221 Tablets

The quality control release method was developed using a single drug product lot rather than various lots with different dissolution profiles and therefore the discriminating power of this method was unknown at the time. As a result, it was difficult to determine whether or not the re-supply lot (Lot 2) was suitable for the clinical studies because there was no quantitative criteria established (i.e., IVIVC) regarding the *in vitro* release profile at this early stage of clinical development.

Referring to Figure 26, the USP apparatus 4 biorelevant dissolution results indicated that Lot 2 is significantly different versus the original supply, Lot 1 with the cumulative percent dissolved for Lot 2 indicating only 60% AMG 221 release versus the original lot. Therefore, these *in vitro* results implied that Lot 2 is not suitable for resupply versus the original lot.

Further investigation of the resupply lot (Lot 2) indicated that while the formulation's composition was consistent between lots the process parameters were modified during the manufacture of the resupply lot (Lot 2) in an attempt to improve the material's flow properties. In particular, the resupply lot was manufactured using a higher percent granulating fluid level and higher impeller speed during the granulating process. See Table 8 for the process parameters for each lot.

Table 8. Processing Parameters of 10 mg AMG 221 Tablets

	Lot 1 (Original Supply)	**Lot 2 (Re-Supply)**
Batch size (g)	4500	4000
Granulating fluid level (%)	30	35
Impeller speed (rpm)	200	300
Fines (< 43 μm)	19.3%	7.2%
%Yield of Compression	84.3%	94.2%
Bulk Density (g/mL)	0.56	0.67
Tap Density (g/mL)	0.69	0.75
Compressibility Index (qualitative flow)	19 (fair)	11 (good)
D10 (μm)	26.4 ± 0.3	65.7 ± 5.4
D50 (μm)	102.2 ± 4.6	194.6 ± 12.7
D90 (μm)	498.3 ± 15.6	716.3 ± 78.6
In-Process Tablet Hardness (kP)	2.1 ± 0.3	2.6 ± 0.2
Tablet Disintegration Time (first to last)	0:45 – 1:21	1:41 – 3:28

kP = N/m^2 = $kg/m/s^2$

These process changes resulted in improved flow properties by making denser granules with larger granule size, however, as a consequence when this material was compressed, the resulting tablets exhibited slower disintegration and dissolution properties versus the original lot (Lot 1).

An *in vivo* crossover study (beagle dog, n = 4) using the original lot (Lot 1) and the re-supply lot (Lot 2) indicated that the re-supply lot had an approximately 70% reduction in maximum concentration (C_{max}) and an approximately 65% reduction in area under the curve (AUC) versus Lot 1. These pharmacokinetic results supported the USP apparatus 4 *in vitro* results that Lot 2 was not suitable for re-supply of AMG 221 clinical materials versus the original lot (Lot 1). Lot 2 was not used to re-supply the clincial study.

Discussion: Case Study V

Lot-to-lot equivalency can be difficult to assess using a quality control *in vitro* dissolution method, particularly when the discriminating power of the method is not fully characterized and an IVIVC has not been established. In this case study, the process parameter changes during the manufacture of the re-supply lot led to slower dissolution using the quality control *in vitro* dissolution release method, where the suitability of the re-supply lot versus the original supply lot was inconclusive.

The *in vitro* biorelevant dissolution method using SIF also indicated a difference in dissolution between the two lots. However, while both methods seem to indicate that the re-supply lot of tablets was not suitable for the clinical study, it was hypothesized that the reduction in C_{max} and AUC for this *in vitro* biorelevant dissolution method may have clinical relevance based on the biorelevant approach during method development. An animal pharmacokinetic

study confirmed that the re-supply lot was unsuitable for the clinical study, giving similar reduction in C_{max} and AUC as was seen in the *in vitro* biorelevant dissolution method. This may be due to the combination of the pH-dependent solubility properties of the compound as well as the different hydrodynamics for USP apparatus 2 and USP apparatus 4.

AMG 221 has a solubility of 0.06 mg/mL, or approximately 6 times sink conditions in 0.1N HCl, the dissolution medium for the USP apparatus 2 method. In contrast, the solubility in the dissolution medium for the USP apparatus 4 method, SIF is 0.037 mg/mL, which is less than 4 times sink conditions. Based on this data, the results from the USP apparatus 4 method appear to have better biorelevant discrimination power.

It is interesting to note that SIF was used directly for AMG 221, a weak base with pKa of 1.5, rather than switching the medium from SGF to SIF, which is the recommendation for weak bases. This may be due to AMG 221's low pKa and the relatively flat solubility curve in this pH range. For other weak bases with higher pKas, a medium switch is still recommended to better reflect the *in vivo* dissolution behavior.

Conclusions

Five case studies were presented to demonstrate the potential applications of this *in vitro* biorelevant dissolution method:

I. Rank ordering of development formulations
II. Effect of pH modifier
III. Assessment and prediction of food effect
IV. Dose proportion
V. Lot-to-lot variability

Case study I demonstrated the application of the *in vitro* biorelevant dissolution method for rank ordering different formulations. This qualitative evaluation of formulations during drug development may significantly help select formulations for further clinical development.

The results from case study II demonstrated the potential to evaluate pH modifiers in a formulation composition using the *in vitro* biorelevant dissolution method. Specifically in cases where a delayed-release strategy (e.g., enteric-coated microgranules) is used, pH modifiers may significantly improve dissolution of the drug at the microenvironment level, which may overcome solubility issues or differences seen during transit through the gastrointestinal tract.

Case study III suggests that the food effect due to bile salt solubility may be assessed *in vitro* using this biorelevant dissolution method with FaSSIF and FeSSIF as the dissolution media. When applied at the appropriate time in a product's development lifecycle, this approach may provide valuable information to understand whether a mitigation strategy is needed to minimize a potential food effect. The results may also facilitate the design of more efficient pharmacokinetic studies or clinical trials later in development.

The results from case study III also suggest that food effect of individual drugs need to be assessed on a case-by-case basis, and it remains challenging to predict the food effect in a reliable fashion since there are many potential causes for a food effect related to a solid oral dosage form (*12*). For example, it is not sufficient to evaluate the presence of a food effect based solely on physicochemical properties of the drug and solubilization capacity of bile salts and surfactants.

Case study IV suggests that it may be possible to assess dose proportion for a drug using the *in vitro* biorelevant dissolution method. However, it is important to note that such evaluations may be limited by the physical constraints of the USP apparatus 4 flow-through cell.

In example of AMG 853 presented in case study IV, the large amounts of drug product material exceeded the limitations of the filter paper therefore disrupting the laminar hydrodynamics of the flow cell, which is meant to mimic the *in vivo* environment. Therefore, it is believed that this application may have has limited utility during drug development.

And finally, the *in vitro* biorelevant dissolution results from case study V indicate the potential to evaluate lot-to-lot consistency using the *in vitro* dissolution method, which is a more common application of the traditional dissolution method. Based on the case study presented, the results from the USP apparatus 4 method appear to have better biorelevant discrimination power versus the traditional USP apparatus 2 dissolution method. This suggests that the *in vitro* biorelevant dissolution method may be able to provide information regarding *in vivo* performance as well as provide the discrimination needed for a quality control method. If this is the case, a fewer number of future PK studies may be needed in the future as greater understanding is gleaned from these *in vitro* biorelevant dissolution studies.

References

1. Prevacid® SoluTabs, www.PDR.net

2. Charman, W.; Porter, C.; Mithani, S.; Dressman, J. Physiochemical and Physiological Mechanism for the Effect of Food on Drug Absorption: The Role of Lipids and pH. *J. Pharm Sci.* **1997**, 269-282.

3. Yu, L. X.; Straughn, A. B.; Faustino, P. J.; Yang, Y.; Parekh, A.; Ciavarella, A. B.; Asafu-Adjaye, E.; Mehta, M. U.; Conner, D. P.; Lesko, L. J.; Hussain, A. S. The Effect of Food on the Relative Bioavailability of Rapidly Dissolving Immediate-Release Solid Oral Products Containing Highly Soluble Drugs. *Mol. Pharmaceutics* **2004**, *1* (5), 357-362.

4. Shono, Y.; Jantratid, E.; Janssen, N.; Kesisoglou, F.; Mao, Y.; Vertzoni, M.; Reppas, C.; Dressman, J. Prediction of Food Effects on the Absorption of Celecoxib Based on Biorelevant Dissolution Testing Coupled with Physiologically Based Pharmaokinetic Modeling. *Eur. J. Pharm. Biopharm.* **2009**, *73*, 107-114.

5. Fang, J. Development of a Biorelevant *In Vitro* Dissolution Method Using USP Apparatus 4 to Predict *In Vivo* Release and Establish IVIVC (Presentation). AAPS National Annual Meeting, 2008, Atlanta, GA.

6. Freston, J. W.; Chiu, Y. L.; Mulford, D. J.; Ballard, E. D. Comparative Pharmacokinetics and Safety of Lansoprazole Oral Capsules and Orally Disintegrating Tablets in Healthy Subjects. *Aliment. Pharmacol. Ther.* **2003**, *17*, 361-367.

7. Amer, F.; Karol, M.; Pan, W.; Griffin, J.; Lukasik, N.; Locker, C.; Chiu, Y. Comparison of the Pharmacokinetics of Lansoprazole 15- and 30-mg Sachts for Suspension Versus Intact Capsules. *Clin. Ther.* **2004**, *26* (12), 2076-2083.

8. Charman, W. N.; Toger, M. C.; Boddy, A. W.; Barr, W. H.; Berger, B. M. Absorption of Danazol After Administration to Different Sites of the Gastrointestinal Tract and the Relationship to Single- and Double-Peak Phenomena in the Plasma Profiles. *J. Clin. Pharmacol.* **1993**, *33*, 1207-1213.

9. Sunesen, V. H.; Vedelsdal, R.; Kristensen, H. G.; Mu¨llertz, A. Effect of Liquid Volume and Food Intake on the Absolute Bioavailability of Danazol, a Poorly Soluble Drug. *Eur. J. Pharm. Sci.* **2005**, *24*, 297-303.

10. Nicolaides, E.; Hempenstall, J. M.; Reppas, C. Biorelevant Dissolution Tests with the Flow-Through Apparatus? *Dissolution Techol.* **2000**, *7* (1), 8.

11. Jantratid, E.; Janssen, N.; Reppas, C.; Dressman, J. Dissolution Media Simulating Conditions in the Proximal Human Gastrointestinal Tract: An Update. *Pharm. Res.* **2008**, *25* (7), 1663-1676.

12. *Guidance for Industry: Food-Effect Bioavailability and Fed Bioequivalence Studies*; U.S. Food and Drug Administration, Center of Drug Evaluation and Research (CDER): Rockville, MD, 2002.

Chapter 4. Conclusions

Overall Conclusions

As previously mentioned, *in vitro* dissolution plays various roles throughout drug development and the traditional QC dissolution method alone may not satisfy the multiple needs for *in vitro* dissolution testing. To that end, one of the most important and commonly used applications of dissolution testing is to predict the *in vivo* performance of solid oral dosage forms. However, there are several limitations of the traditional QC dissolution method, including inadequate dissolution of poorly soluble drugs as well as the use of simple aqueous buffer solutions and hydrodynamics, which do not represent the *in vivo* environment.

The *in vitro* biorelevant dissolution method developed addressed some of these limitations by using biorelevant dissolution media and equipment (i.e., USP apparatus 4 open system) with optimized instrument parameters (e.g., glass beads, flow rate, flow cell size and design, etc.) to mimic the hydrodynamics *in vivo* in a qualitative manner.

The work presented in this thesis suggests there are several potential applications for the *in vitro* biorelevant dissolution method developed, particuarly for BCS Class II compounds. These applications include rank ordering of formulations, evaluation of pH modifiers, evaluation of food effect, evaluation of dose assessment, and lot-to-lot consistency.

Considerations for Future Work

While there are several applications of the *in vitro* biorelevant dissolution method developed, there are also several potential limitations for the widespread use of this method. One apparent, but not trivial limitation is the difficult set-up and use of the system. Specifically,

there are several intricate parts to the assembly of the flow cell, which are not only tedious to put together, but the process may be difficult to remember after a long period of disuse. With this in mind, it would be recommended to have a dedicated person(s) to run this system to ensure consistency from experiment to experiment.

Additionally, it is important to note that all of the cases described in this thesis work are of BCS class II compounds, where dissolution is a rate-limiting step in the drug absorption process. With this in mind, it is possible that the constraints of this thesis work may have contributed to these findings including the use of:

- systematic method development approach guided by known *in vivo* profiles,
- USP apparatus 4 open system that simulates *in vivo* hydrodynamics and continuously removes the dissolved material to maintain sink conditions,
- biorelevant dissolution media that mimics the *in vivo* GIT environment,
- and physicochemical characteristics of BCS class II compounds

More work is this area is needed to further understand and explain these empirical observations, which may be an opportunity for integration of simulation work to explain a drug's absorption behavior. While this was of interest during the thesis research, there was not adequate time to learn the simulation software to yield fruitful results.

Ultimately, this *in vitro* biorelevant dissolution method yielded some interesting qualitative results, however may be limited in its utility in a quantitative nature due to some of the limitations described. With this in mind, in some cases USP apparatus 2 with biorelevant dissolution media may still be more straightforward to evaluate qualitative relationships during preliminary *in vitro* biorelevant dissolution.